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Primary Immunodeficiency Diseases

Report of an IUIS Scientific Committee*

1 INTRODUCTION

The initial barriers to infection are skin, mucous membranes and the substances they secrete. When infectious agents penetrate these barriers other nonspecific host factors such as cytokines and complement come into play. These components, together with the specific immune mechanisms of antibodies and lymphocytes, constitute the immune system. The complex of interacting factors and cells provides the initial innate nonspecific defence and subsequently the acquired specific defence mechanisms for resistance to infection.

The primary immunodeficiency diseases result from innate defects of the immune system. As a consequence, recurrent protozoal, bacterial, fungal and viral infections of varying severity ensue. The immune system can also be adversely affected secondarily by a variety of pathological conditions (including malignancy, metabolic diseases and malnutrition) and drugs; these result in secondary immunodeficiencies.

Both primary and secondary immunodeficiencies result in a similar spectrum of illness: recurrent or persistent infections. As the relationship between immunity and infection is interactive, infection may cause as well as result from immunodeficiency. Many infectious agents, including the human immunodeficiency virus (HIV), have both specific and nonspecific effects on the immune system.

Study of patients with primary immunodeficiency diseases has expanded our understanding of immunity. Recent progress in immunobiology and genetics has, with increasing precision, identified the causes of many of the Primary Immunodeficiency Diseases; diagnosis and therapy can, as a result, be more specific and effective.

2 CELLULAR BASIS OF THE IMMUNE RESPONSE

The progenitors of T cells, B cells and natural killer (NK) cells are derived from the same multipotent haematopoietic stem cells (HSC) that give rise to other types of blood cells. Cells of the monocyte–macrophage series, including Langerhans cells and dendritic cells, process and present antigen to both the T and B cells both early in their development and later after they reach maturity (see Fig. 1).

Progenitor cells migrate from the circulation into the epithelial thymus where they interact with the stromal cells and their soluble products to undergo cell division, clonal selection and maturation. The T-lineage cells interact with their microenvironment through cell surface glycoproteins that serve as adhesion molecules and receptors coupled to signal transduction elements. An early thymocyte decision determines the choice of one of two pathways of differentiation. Progenitor cells (pro-T cells) may rearrange and express $\gamma\delta$ T-cell receptor (TCR) genes together with the CD3 complex of proteins to become $\gamma\delta$ T cells. Alternatively, precursor cells may rearrange their VDJβ genes and express the completed β chain together with a pre-T α chain (pTα) and the CD3 protein signalling complex. These pre-T cells then rearrange their VJα genes to produce α chains and become $\alpha\beta$ T cells. Cells of this lineage (immature T cells) initially express both CD4 and CD8 molecules that interact, respectively, with MHC class II or class I molecules on thymic stromal cells to influence their maturation into $CD4^+$ or $CD8^+$ T cells. Positive or negative selection of immature $\alpha\beta$ T-cell clones is determined by the affinity of the TCR interaction with self-antigens presented as peptide fragments within the grooves of MHC class II and/or class I molecules on thymic stromal cells. The $\gamma\delta$ T cells do not express CD4 or CD8 molecules during their intrathymic maturation, and intrathymic clonal selection is probably not essential for their development. The $\gamma\delta$ T cells can be subdivided on the basis of their utilization of either the γl or γ2 constant region genes together with preferred sets of VDJδ genes.

T-cell development in the thymus requires integrity of each of the TCR/CD3 components, CD4, CD8, certain cytokines, cytokine receptors, and their signal transduction partners. Later, when they migrate to the periphery, T cells may undergo selective clonal activation leading to proliferation and maturation. Antigen activation involves the interaction of T-cell receptors with antigen fragments held within the grooves of MHC class I or class II molecules. The activated $\alpha\beta$ T cells begin to produce lymphokines such as IL-2 and express high-affinity receptors for this lymphokine. The interaction of IL-2 with its receptor modulates T-cell growth and effector function.

The role of $\gamma\delta$ T cells is presently unclear, but their acquisition of CD8 in peripheral tissues may enhance interaction with target cells bearing class I (or class I-like) MHC gene products. There is increasing evidence that $\gamma\delta$ cells require exogenous growth factors, such as IL-7, produced by $\alpha\beta$ T cells or other cell types. Crosstalk between $\alpha\beta$ and $\gamma\delta$ T cells may co-ordinate their activities to control immune responses.

The development of B lineage cells is a multifocal process which is concentrated in fetal liver before bone marrow becomes the major haematopoietic organ. Progenitor cells (pro-B cells) receiving signals from local stromal cells begin to divide, rearrange their immunoglobulin VDJ gene segments, and give rise to clones of pre-B cells. The pre-B cells express low levels of receptors composed of μ heavy chains, a surrogate light-chain complex of V

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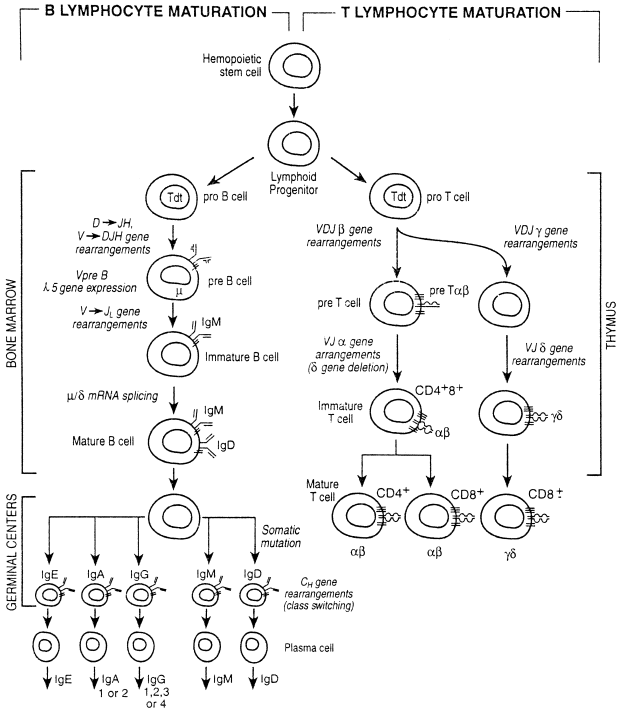

Fig. 1. Development of T and B lymphocytes.

pre-B and λ-5 proteins, and an Igα/Igβ dimer. The cytoplasmic tails of the Igα and Igβ chains contain immunoregulatory tyrosine activation motifs (ITAMs) that are needed for signal transduction. Pre-B cells, thus equipped, may then rearrange VJ gene segments in their light chain loci to become immature IgM^+ B cells. Immature B cells are easily tolerized or killed by premature stimulation via their antigen receptors. After migrating from the bone marrow, the B cells mature, express IgD antigen receptors, and respond to antigens and $CD4^+$ T-cell help by undergoing proliferation and plasma cell differentiation.

In germinal centres, B cells see antigen on follicular dendritic cells and interact with helper T cells to undergo proliferation, somatic mutation and Ig class switching. Germinal centre B cells that produce antibodies of relatively high antigen affinity are selected to give rise to plasma cells that produce different Ig isotypes (IgM, IgG, IgA or IgE) or become recirculating memory B lymphocytes. Cell interaction molecules important for the germinal centre response include complement receptors and CD40 on B cells (and dendritic cells) and the CD40 ligand on activated T cells. The activated B cells express CD80 and CD86 molecules that, in turn, interact with CD28 and CTLA molecules on T cells to modulate their response. Cytokines (IFNγ, IL-2, 4, 5, 6, 7, 10, and 12–15) and their cell surface receptors are also important in facilitating the genetically restricted interactions between antigen-presenting cells and T cells to elicit cell-mediated immunity, and the $CD4^+$ T-cell interaction with B cells required for humoral immunity. CD4+ helper T cells (Th) are directed by different cytokines along two functionally distinct pathways, Th1 and Th2. Th1 helper T cells characteristically produce cytokines that enhance inflammation, IL-2, IFNγ and TNF, whereas Th2 helper T cells produce cytokines that enhance antibody production, namely IL-4, IL-5, IL-10 and IL-13. IL-12 and IFNγ facilitate the Th1 cell differentiation pathway involved in cell-mediated inflammatory responses. Conversely, IL-4 facilitates the Th2 pathway of cell differentiation to yield helper T cells that promote the antibody response. In parallel with the CD4 cells, there are two groups of CD8 cytotoxic T cells, Tc1 and Tc2, each of which produces distinct sets of cytokines.

The basic cellular elements of the immune system are well established by 15 weeks of human gestation. Nevertheless, the system is functionally immature at birth and requires antigen selection and experience to achieve full maturation during infancy.

3 GENETIC BASIS OF THE IMMUNE RESPONSE

Immunoglobulins, which are tetramers of two heavy and two light chains, serve as antigen receptors on B cells, and the secreted antibodies are the effectors of the humoral immune system. Heavy chains of immunoglobulins are encoded by genes on chromosome 14 at band q32, whereas the genetic locus of kappa light chain genes is on chromosome 2p11 and of lambda on chromosome 22q11 (Fig. 2). The variable domains of immunoglobulins are encoded by discontinuous gene segments that are separated from each other in the germline state (Fig. 2). The heavy-chain gene family consists of approximately 50 variable-region (V_H) genes that encode the first 95 amino acids of the variable portion of this peptide, more than 20 diversity-region (D) genes that encode a small number of amino acids, six joining-region (J_H) genes that encode the remaining 13 amino acids of the variable region, and nine functional constant-region (C_H) genes. The κ and λ gene families also contain a series of variable-region and joining genes located upstream from the constant-region gene or genes. As the pluripotent stem cell with its immunoglobulin genes in the separated germ-line configuration develops into an immunoglobulin-producing plasma cell, a process of DNA rearrangement occurs. This begins with activation of a heavy-chain gene by a rearrangement that combines a single D segment with a single J_H segment. Then a single V_H segment is combined with this DJ juncture. This rearrangement of a V gene segment with a D gene segment brings a promoter-controlling sequence upstream from each V gene segment closer to a tissue-specific enhancer sequence that is between the J and C regions. This activates the gene complex, increasing transcription of mRNA for the heavy-chain gene, and leads to the production of cytoplasmic μ chain and, thus, the appearance of the

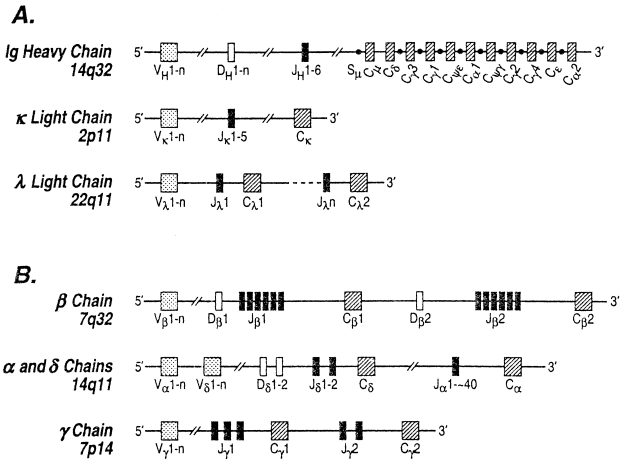

Fig. 2. (a) Genes of immunoglobulin chains and (b) T-cell receptor chains.

pre-B cell. Surrogate light-chain genes, V pre-B and λ-5, are expressed without rearrangement during the earliest stages of B lineage differentiation. The products of these genes become associated with the μ heavy-chain gene product and appear on the surface of pre-B cells. Following effective heavy-chain gene rearrangement, there is a rearrangement of light-chain genes, beginning with rearrangements of the κ immunoglobulin locus with a recombination that juxtaposes one of many V_κ regions with one of the five J_κ segments to generate the complete transcriptionally active V_κ region. If efforts at generating a κ gene are not successful, activation of λ light-chain genes occurs. Following effective rearrangement of light-chain genes, the mature mRNA is translated, and IgM molecules can be produced and expressed on the cell surface, thus producing the immature B cell.

Although a B cell and its progeny produce only a single form of light chain, κ or λ, a B cell is capable of simultaneously producing IgM and IgD membrane forms of immunoglobulin and of switching, subsequently, to the production of other immunoglobulin isotypes. Establishing the order and structure of the heavy-chain constant region genes has helped to elucidate the mechanisms by which different classes are produced. The human immunoglobulin heavy-chain constant-region genes located on the long arm of chromosome 14 at band q32 are in the order shown in Fig. 2. The simultaneous production of IgM and IgD membrane forms, as well as the transition from membrane-bound receptors to a secreted form, involves alternative mRNA splicing. In contrast, the transition from a $C_{\mu\delta}$-expressing B cell to one expressing another isotype occurs by a phenomenon known as heavy-chain class switching or isotype switching. This is accomplished by the splicing of an area termed a switch region upstream from the μ heavy-chain gene, with the switch region 5′ to the downstream heavy-chain gene to be expressed. Such recombination would result in a DNA rearrangement that is accompanied by deletion of the DNA between the switch region 5′ from the C_μ gene and the switch region immediately 5′ from the constant region to be used. This process of switching allows a new constant region to be transcribed with the pre-existing $V_H/D/J_H$ recombined gene. In addition, both membrane and secreted forms of the immunoglobulins may be produced by the same cell at different stages of differentiation. At a molecular level, the transition from the membrane to the secreted form involves alternative splicing of mRNA resulting in different mRNAs containing the secreted ($C\mu$s) or membrane ($C\mu$m) carboxy-terminal tail. Terminal differentiation of a B lymphocyte to a plasma cell forecloses these options so that a single plasma cell synthesizes and secretes an immunoglobulin of a single isotype and specificity (i.e. allelic exclusion).

The mature B-cell antigen complex is composed of an antigen-binding membrane immunoglobulin and the associated Igα/β proteins serving transducer/transporter functions. The transducer/transporter substructure is composed of disulphide-linked heterodimers of Igα (CD79a) and Igβ (CD79b) subunits. Igα is a product of the mb-1a gene and Igβ is encoded by the B29 gene. Thus, the pre-B receptor complex involves a minimum of 10 chains: two Ig heavy chains, two Vpre-B chains, two λ5 chains and two Igα/β heterodimers. The CD19 complex that includes CD19, complement receptor 2 (CR2 or CD21), Leu-13 and TAPA-1 molecules may act as a coreceptor with the B-cell antigen receptor binding to the same antigen/complement complex.

The T-cell receptors for antigen are also heterodimers composed of either α and β or γ and δ subunits. The T-cell antigen receptor is associated with a cell surface complex of different nonpolymorphic chains (CD3γ, CD3δ, CD3ϵ, CD3ζ). The arrangement of T-cell receptor genes is similar to that of Ig genes (Fig. 2). The T-cell receptor TCRβ chain locus is on chromosome 7q32–34, and the TCRγ chain is on chromosome 7p14–15. The TCRα and δ genes are on chromosome 14q11. The TCRβ chain gene comprises discontinuous germ-line variable regions gene segments (Vβ) and duplicate sets of diversity (Dβ1, Dβ2), joining (Jβ1, Jβ2) and constant (Cβ1, Cβ2) gene segments. The TCRα gene consists of multiple variable (Vα) genes arranged in families, at least 40 joining (Jα) genes in a tandem array and a single 5′ constant Cα gene. The TCRδ gene system composed of Vδ, Dδ, Jδ and Cδ segments is nested within the TCRα locus between the TCRα variable and TCRα joining region genes. The fourth gene family, the TCRγ family encoded by genes on the short arm of chromosome 7 (7p15), has many properties in common with other TCR genes, including assembly from diverse variable, joining and constant regions and rearrangement in T cells.

As with Ig genes, there appears to be a hierarchy in the rearrangement and expression of T-cell receptor genes. Rearrangement of the TCRγ and δ genes occurs early. If the rearrangements are effective, the $\gamma\delta$-T-cell receptor subunits together with the CD3 complex of proteins are expressed on the cell surface of T$\gamma\delta$ cells. In an alternative pathway of rearrangement, precursor cells initiate rearrangements of the TCRβ genes. In analogy with λ5-IgH pre-B-cell receptors there is a pre-T-cell receptor that is involved in the regulation of early T-cell development. The pre-T-cell receptor involves CD3 complex noncovalently associated with the TCRβ chain that in turn is disulphide linked to a pre-T-cell receptor α chain (pTα), a type I transmembrane protein that acts as a surrogate for the TCRα chain. Subsequently, TCRα genes are rearranged and expressed, permitting the production and cell-surface expression of the α/β heterodimer. The mature T-cell receptor heterodimers become associated with the CD3 complex which serves a transporter/transducer function. The receptor complex is then expressed on the surface of T cells. CD4 or CD8 act as coreceptors for the TCR by binding to the same MHC molecules as the TCR.

4 CYTOKINES AND CHEMOKINES

Immune responses as well as the effector phase of immune reactions are regulated by soluble mediators called interleukins or cytokines. Many cytokines and their receptors have been characterized in molecular form. Characteristic features of cytokines are their functional pleiotropy and redundancy, i.e. one cytokine shows multiple functions in a wide variety of tissues and cells and many different cytokines exert similar effects in the same cells. Cytokine producers are also multiple, i.e. many cytokines are produced by several different cells, and the production of cytokines is influenced by other cytokines, thus forming a 'cytokine network'. Major producers of cytokines in the immune system are monocytes and T cells.

Many cytokine receptors belong to the 'cytokine receptor family'. They have four conserved cysteine residues in their N-terminal region and a 'Trp–Ser–X–Trp–Ser' motif external to the plasma membrane. These conserved residues are essential for maintaining the tertiary structure of the receptor molecules. Cytokine receptors do not have any unique sequences for signal transduction, such as tyrosine kinase, in their intracytoplasmic domain. Several cytokine receptors, such as IL-2Rα, IL-6R, IL-5R, GM-CSFR have very short intracytoplasmic domains, suggesting the presence of other chains for signal transduction. The IL-6

receptor system was shown to be composed of two polypeptide chains, an 80-kDa IL-6R and a 130-kDa signal transducer (gp130). Binding of IL-6 to IL-6R triggers an association with the 130 kDa subunit which transduces the signal. gp130 has been shown to function as a signal transducer not only for IL-6 but also for LIF, Oncostatin M, IL-11 and ciliary neurotropic factor (CNTF) and cardiotropin (CT-1). Thus, this cytokine receptor system consists of two polypeptide chains, ligand-specific receptor and common signal transducer. Recently, this concept has been shown to be applied to most other cytokine receptor systems. In the haematopoietic system, the receptors for IL-3, IL-5 and GM-CSF utilize a common β chain (βc) as a signal transducer. In the lymphoid system, a common γ chain (γc) is a shared element of the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15. This is a reason why mutation in the gene encoding γc results in X-linked SCID in humans, whereas a disruption of the IL-2 gene in mice did not lead to a major effect on the development of T and B lymphocytes. IL-4 and IL-13 receptors share a common 140-kDa heavy chain (IL-4Rα) which associates with the γc in IL-4R and with a novel IL-13-binding chain of the IL-13R.

A mechanism common to a very large number of cytokine receptors which consist of two or more chains involves the cross phosphorylation and activation of members of the Jak kinase family. Cytokine receptors associated with Jak family kinases include receptors for IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15 as well as for GM-CSF, IFNα, IFNβ and IFNγ. Cytokine binding induces receptor chain heterodimerization. This results in cross-phosphorylation on tyrosine residues and activation of the associate Jak kinases (e.g. Jak1 associated with IL-4R-α and Jak3 associated with γc). Activated Jak kinases phosphorylate tyrosine residues on one, or more, of the receptor chains. These P-Tyr residues then serve as docking sites for SH2 domain-mediated docking of STAT (signal transduces and activators of transcription) molecules, e.g. STAT6 in the case of IL-4R. Receptor-bound STAT molecules are, in turn, tyrosine phosphorylated. This leads to their dimerization through P-Tyr–SH2 chain interactions, uncovering nuclear localization signals. STATs then translocate to the nucleus where they bind to the DNA consensus sequence [TTC NNN (N) GAA] and participate in the transcriptional activation of cytokine responsive genes, e.g. Cϵ in the case of IL-4.

At present, 18 interleukins (IL-1 to IL-18) have been cloned and their biological activities in immune regulation are under intense scrutiny. Assays are available for estimation of cytokine levels.

4.1 IL-1

IL-1 is one of the typical examples of multifunctional cytokines. It is produced mainly by monocytes. IL-1α and IL-1β, which show only 24% (human) homology in their amino acid sequences, utilize the same IL-1 receptor, which belongs to the Ig-superfamily. A naturally occurring IL-1 inhibitor (IL-1ra) also shows a certain sequence homology with IL-1α and β and binds to the IL-1 receptor, but it cannot activate the signal pathway. Therefore, IL-1ra functions as a competitive inhibitor of IL-1. IL-1 is important for the early activation of T cells as a costimulatory factor. IL-1 is a strong inducer of IL-6 and several activities of IL-1 in immune regulation can be exerted through IL-6. IL-1 is one of the typical inflammatory cytokines and is involved in the generation of prostaglandins. Fever is generated by IL-1 through the generation of IL-6.

4.2 IL-2

IL-2 is a major T-cell growth factor. Activated T cells produce IL-2 and express high-affinity IL-2 receptors, and T cells proliferate in an autocrine or paracrine fashion. IL-2 is not needed for T-cell maturation as both humans and mice with IL-2 deficiency possess a normal number of circulating T cells, which, however, fail to proliferate to mitogens and antigens unless IL-2 is added. IL-2 plays an important role in mature T-cell apoptosis (activation-induced cell death; AICD). The high-affinity IL-2 receptor is formed by three polypeptide receptor components, IL-2Rα (Tac, CD25), IL-2Rβ, and γc and signals can be transduced through IL-2Rβ and γ chains. Activated B cells can express IL-2R and IL-2 induces growth and antibody production in such activated B cells. Resting NK cells express IL-2Rβ and γc. The IL-2Rβ chain associates with Jak1 and γc associates with Jak3. Engagement of the IL-2R results in phosphorylation and activation of STAT 5. Mutation in the γc chain gene or mutation of Jak3 result in phenotypically similar severe combined immunodeficiency with absent T and NK cells, but circulating B cells.

4.3 IL-3

IL-3 is a multicolony stimulatory factor (multi-CSF) and is involved in proliferation of early progenitors of haematopoietic cells. IL-3 exerts a synergistic effect with IL-6 on the expansion of early haematopoietic progenitors. IL-3 is produced by activated T cells. The IL-3 receptor comprises two polypeptide chains similar to the IL-6 receptor. The βc chain that functions as a signal transducer for the IL-3 receptor is common to IL-5R and GM-CSFR. These three receptors use the Jak1/Jak2 and STAT 5 pathway.

4.4 IL-4

IL-4 was originally identified as B-cell stimulatory factor (BSF-1) and was shown to induce the early activation of resting B cells upon antigen exposure. IL-4 induces isotype switching of B cells into IgE-producing cells. Anti-IL-4 inhibits IgE production in parasite-infected mice, indicating an essential role for IL-4 in IgE production. IL-4 knockout mice do not produce any IgE. IL-4 is shown to be a potent growth factor for mast cells and to induce FcϵRII (CD23) on B cells and monocytes. These results strongly suggest the involvement of IL-4 in immediate-type hypersensitivity. IL-4 is produced not only by activated T cells but also by mast cells and basophils. IL-4 functions as a growth factor for T cells and is involved in autocrine and paracrine growth of activated T cells. The IL-4 receptor consists of a heavy 140 kDa chain which associates with Jak1 and of the γc chain which associates with Jak3 and acts as a signal transducer. IL-4R engagement leads to the phosphorylation and activation of STAT 6.

4.5 IL-5

IL-5 may enhance B-cell differentiation and also acts as an eosinophil differentiation factor. The IL-5 is mainly produced by activated T cells. The IL-5 receptor consists of two polypeptide chains similar to the IL-6R, one of which (βc) is a signal transducer of IL-5R that is common to GM-CSFR and IL-3R. IL-5 knockout mice fail to develop eosinophilia.

4.6 IL-6

IL-6 is the prototypic multifunctional cytokine. It was originally identified as a B-cell differentiation factor and is involved in the final maturation of B cells into antibody-producing cells. IL-6 is

essential to antibody production. It also activates T cells and haematopoietic progenitors. IL-6 induces maturation of megakaryocytes and induces thrombocytosis in inflammation. IL-6 is a major inducer of the acute-phase reaction in inflammation. Excessive production of IL-6 has been shown in several autoimmune diseases. IL-6 is a potent growth factor for myeloma and plasmacytoma cells and appears to be involved in multiple myeloma and plasmacytomas. The IL-6R consists of an IL-6-binding chain and a signal-transducing gp130 chain shared by receptors LIF, CNTF and OSM. The IL-6 uses the Jak1/Jak2 and STAT3 pathway. IL-6 is produced by a wide variety of cells, but mainly by monocytes. IL-1 and TNFα are strong inducers of IL-6 in monocytes. Antiviral antibody response was 5–10-fold reduced in IL-6 knockout mice. Furthermore, IL-$6^{-/-}$ mice were defective in their mucosal IgA response. The inflammatory acute-phase reaction is severely compromised in IL-$6^{-/-}$ mice; optimal responses to trauma and infection can only be mediated in the presence of IL-6.

4.7 IL-7

IL-7 is a major B-cell lymphopoietin and is involved in the growth and differentiation of pre-B cells. It also acts as a growth factor for thymocytes and mature CD4+ and CD8+ cells. IL-7 is produced by stromal cells in the marrow, thymus and spleen. IL-7R utilizes γc in signal transduction. Lymphoid development is severely impaired in IL-$7^{-/-}$ and IL-$7R^{-/-}$ mice.

4.8 IL-8

IL-8 is an inflammatory cytokine produced by monocytes and involved in neutrophil chemotaxis. Several other cytokines, such as platelet basic protein (PBP), platelet factor 4 (PF4), γ-interferon inducible protein (IP10) and growth related gene (Gro), show sequence homology with IL-8. IL-8, PBP, PF4, IP10 and Gro belong to the CXC family of chemokines. The other family of chemokines is the CC family. In contrast to CC chemokines which have two adjacent cysteines, CXC chemokines have the same two cysteines separated by an amino acid. The list of chemokines is currently expanding with more than 20 family members, some of which overlap in function, receptor utilization and target specificity. Recently, the CCRK5 receptor was shown to be shared by the CC chemokines Rantes, Mip1-α and Mip1-β and to function in monocytes as a coreceptor with CD4 for the HIV gp120. In parallel, the CXC chemokine receptor fusin (CXCR3) is the coreceptor with CD4 for HIV entry into T cells. The CXC chemokine that uses CXCR3 as its receptor is SDF-1/PBSF. This stromal-derived factor/pre-B cell stimulatory factor shows synergy with IL-7 for B-cell development. Knockout of SDF-1/PBSF results in failure of B-cell development.

4.9 IL-9

IL-9 is identified as a T-cell growth factor distinct from IL-2 or IL-4. It is produced by CD4+ helper T cells and acts on helper T cells but not on CD8+ cytotoxic T cells. IL-9 was shown to act on mast cells, stimulating their growth in a manner similar to IL-4. IL-9R utilizes γc in signal transduction.

4.10 IL-10

IL-10 was originally called CSIF (cytokine synthesis inhibitory factor), which is produced by monocytes and Th2 cells. It inhibits the production of cytokines by Th1 cells. As with other cytokines, IL-10 also exerts pleiotropic functions and induces growth of T cells and mast cells. IL-10 is produced not only by Th2 cells but also by B lymphoma cells, macrophages and mast cells. The IL-10 receptor is composed of two chains and uses the Jak1/Tyr2 and STAT 3 pathway.

4.11 IL-11

IL-11 is identified as a plasmacytoma growth factor and has the pleiotropic functions of IL-6 and its receptor shares the gp130 of the IL-6R.

4.12 IL-12

IL-12 is a heterodimer of glycoproteins, p35 and p40, which acts on B cells, NK cells and monocytes to induce proliferation and cytokine synthesis, especially of interferon-γ. IL-$12^{-/-}$ mice show increased susceptibility to leishmaniasis. IL-12R uses the Jak2/Tyr2 and the STAT4 pathway.

4.13 IL-13

IL-13 is produced by Th2 and mimics the effects of IL-4 on IgE production. The IL-13R is expressed on human B cells, T cells and monocytes. It uses a unique IL-13-binding chain which associates with Jak2 and shares with the IL-4R the 140-kDa IL-4Rα chain which associates with Jak1. IL-13 activates STAT-6.

4.14 IL-14

IL-14, formerly called high-molecular-weight B-cell growth factor, has been reported to enhance growth and differentiation of B cells. The existence of this cytokine has been challenged.

4.15 IL-15

IL-15 acts on activated T cells, B cells, and on NK cells to induce proliferation and differentiation. Its major receptor includes IL-2Rβ and γc as well as a unique IL-15R α chain. An alternative IL-15 receptor is found on mast cells.

4.16 IL-16

IL-16 is a CD4+ T lymphocyte attractant and competence growth factor which uses CD4 as a receptor. CD8+ T cells serve as a source of IL-16.

4.17 IL-17

IL-17 is a glycoprotein of 155 amino acids secreted as a homodimer by activated memory CD4+ T cells, and is highly homologous to Herpesvirus Saimiri gene 13. IL-17 stimulates epithelial, endothelial and fibroblastic cells to secrete cytokines such as IL-6, IL-8 and granulocyte-colony-stimulating factor, as well as prostaglandin E_2. Furthermore, when cultured in the presence of IL-17, fibroblasts could sustain the proliferation of CD34+ haematopoietic progenitors and their preferential maturation into neutrophils.

4.18 IL-18

IL-18 is a novel cytokine produced by liver cells that possesses potent biological activities, including the induction of IFN-γ production by spleen cells and the enhancement of NK cell cytotoxicity. In addition, IL-18 augments granulocyte–macrophage–CSF production and decreases IL-10 production by Con A-stimulated PBMC.

4.19 Other cytokines

In addition to the interleukins and their receptors, other cytokines and monokines affect the immune system. Interferon-γ, secreted by activated T cells, is the most important cytokine in the induction of MHC class II molecule expression. TNF-α is a prominent

monokine that shares many functions of IL-1, except for the induction of IL-2. TNFαR are of two types. TNFRI mediates cell death and uses signal transducer molecules that contain death domains. TNFRII delivers growth signals and activates NFκB. It uses TRAF1 and TRAF2 molecules as signal transducers. Lymphotoxin α-β is important for the normal development of lymphoid architecture (in mice). The colony-stimulating factors, such as GM-CSF, G-CSF and M-CSF, act in addition to IL-3, as growth factors for immunologically relevant cells. The interferon α, β and γ receptors use the Jak/STAT signalling pathway. Deficient interferon-γ receptor due to mutations in the α chain in the receptor results in undue susceptibility to intracellular pathogens that reside in macrophages and in fatal mycobacterial infection. Immunity to infection with *Listeria monocytogenes* is severely compromised in TNFR$^{-/-}$ as well as interferon-$\gamma^{-/-}$ and IL-6$^{-/-}$ mice, indicating an important role of these cytokines in innate immunity. Transforming growth factor β (TGFβ, a cytokine produced by many cells) has been identified as a major suppressor of immune function and is involved in class switching to IgA.

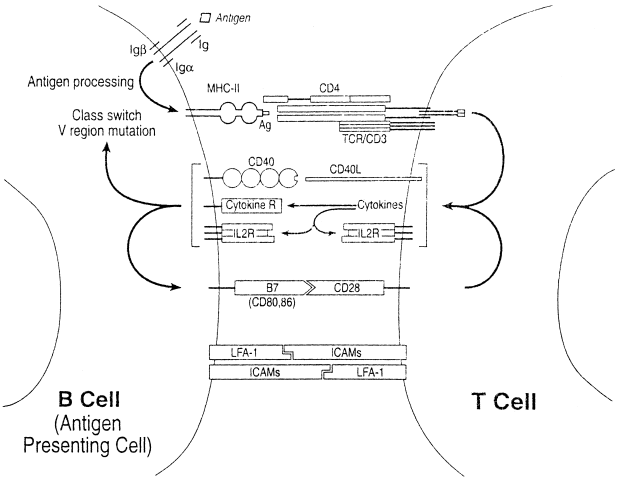

Fig. 3. Adhesion molecules and their ligands.

5 ANTIGEN PRESENTATION, CELL ADHESION AND SIGNAL TRANSDUCTION

Antigens are taken into antigen presenting cells (APC) by receptor-mediated endocytosis (via C3 or Ig receptors) or by fluid-phase endocytosis. Protein antigens, once in the phagolysosome, are digested by proteolytic enzymes. Antigenic fragments are then shunted to a specialized compartment for peptide loading. In this compartment, antigenic peptides bind to class II histocompatibility molecules and the complex thus formed is transported to the cell surface of the APC. For the most part, cells of monocyte–macrophage lineage and mature B cells serve as APC.

For antigens within the cell, including many viral antigens, there is degradation of the proteins in part by proteasomes followed by transport of the resultant peptides into the endoplasmic reticulum where they bind to class I MHC. This transport across the Golgi membrane is mediated by members of the ATP-binding cassette (ABC) superfamily of transporters (e.g. TAP-1 or TAP-2), which are encoded by genes within the MHC. Mutations in TAP-2 are associated with MHC class I deficiency.

The interaction of APC with T cells as well as T–B-cell collaboration are facilitated by adhesion molecules. The ligands and counter-ligands of many of these adhesion molecules have now been well defined (see Fig. 3). ICAM-1 and LFA-1 are reciprocally interacting; both molecules are found on T cells as well as on APC. LFA-1 is defective in leucocyte adhesion deficiency (see 12.2.1) and the CD40 ligand is defective in the hyper-IgM syndrome (see 9.2.2).

The interaction of the T-cell receptor (TCR)–CD3 complex with presented antigen results in signal transduction that leads to phosphorylation of CD3 and the activation of T cells (see Fig. 4). Upon TCR crosslinking, the src type protein tyrosine kinases (PTK), p56^{lck} and p59^{fyn}, respectively, associated via their N terminal domains with CD4 and CD3ζ, phosphorylate CD3ζ in tyrosine residues of the immune tyrosine activation motif (ITAM),Y-X-X-L-X(7–8)-Y-X-X-L/I. Three ITAMs are present in CD3ζ. Activation of lck and fyn also critically depends on the transmembrane phosphatase CD45, which dephosphorylates the carboxy-terminal autoinhibitory tyrosine residue of these src kinases. The phosphorylation of CD3ζ initiates an activation cascade by enabling the TCR to recruit downstream molecules. Thus, phosphorylated CD3ζ is now able to bind the PTK, ZAP-70, and the adaptor protein, Shc, via interaction between phosphotyrosine residues on CD3ζ and SH2 domains in these proteins. Receptor-bound ZAP-70 can phosphorylate substrates such as phospholipase C-γ (PLC-γ) and recruit them to the receptor complex. The other CD3 components, each of which contains one ITAM motif, may also serve as substrates for p56^{lck} and p59^{fyn} and, as demonstrated for CD3ϵ, may bind ZAP-70 and thus may function as additional independent signalling units.

Phosphorylated PLC-γ recruited to the membrane breaks down membrane inositol phosphatides, mainly PI4, 5-P2, to generate the second messengers diacylglycerol (DAG) and inositol triphosphate (IP3) which, respectively, activate protein kinase C (PKC) and mobilize Ca^{++} from intracellular stores. Released Ca^{++} plays a critical role in the activation of downstream enzymes, which include the Ca^{++}/calmodulin-dependent phosphatase, calcineurin. Activated calcineurin dephosphorylates nuclear factor of activated T cells (NFAT) allowing its translocation to the nucleus. Several isoforms of NFAT exist, of which NFATc is the most critical for T-cell activation. Grb-2 interacts with, among other things, Vav, which is phosphorylated by ZAP-70 following TCR receptor

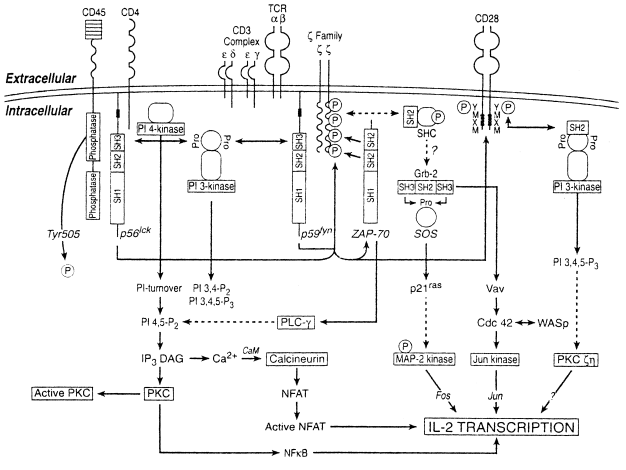

Fig. 4. Signal transduction pathway in T cells. CD3 is indiated in the T-cell lineage by 5 bars and Igα and Igβ of the B cell receptor by two bars.

engagement. Vav is a GTP exchange factor that activates the GTPase, Cdc42, a member of the rho family of proteins. In its GTP-bound state, Cdc42 binds Wiskott–Aldrich syndrome protein (WASp) and activates the Jun kinase pathway. Jun and Fos form a heterodimer that can bind to specific DNA sequences as well as to NFAT to enhance its transcriptional activity.

Another activation pathway recruited by aggregation of the TCR is the $p21^{ras}$ pathway. Phosphorylated CD3ζ has been reported to bind to the SH2 domain-containing adaptor protein, Shc. Shc protein activates $p21^{ras}$ by means of the intermediate adaptor protein Grb-2, consisting of two SH3 and one SH2 domains, and the guanine nucleotide releasing protein SOS, which is capable of exchanging GDP with GTP leading to the conversion of $p21^{ras}$ to an activated GTP-bound state. This in turn activates the MAP kinase pathway, which culminates in nuclear fos/jun expression.

In addition to the tyrosine phosphorylation cascade, the activated lipid kinases, PI-3 kinase and PI-4 kinase, are recruited to the activated TCR. PI-3 kinase is recruited by association of its p85 noncatalytic subunit with the SH3 domain of $p56^{lck}$, $p59^{fyn}$ or ZAP-70. PI-3 kinase phosphorylates the D-3 position of the inositol ring of phosphatidylinositol leading to the generation of PI-3,4-P2 and PI-3,4,5-P3. PI-3,4,5-P3 activates PKC isozymes, e.g. PKCζ, which play an important role in cell survival and division.

Activation of PKC, calcineurin and ras and Cdc42 result in the activation and expression of a number of transcription factors that include, respectively, NFκB, NFAT, Fos and Jun. These factors are critical for the transcription of *IL-2* and *CD40 L*. The expression of these genes leads to T-cell activation and proliferation.

Optimal *IL-2* gene expression requires, in addition to TCR crosslinking, engagement of the costimulatory molecule CD28 by its counter-receptor B7 (CD80 and CD86) on APCs. CD28 contains a YMXM motif, which is a potential target for phosphorylation by PTK that has been activated following TCR crosslinking. The phosphorylated motif recruits PI-3 kinase via the SH2 domain of its noncatalytic subunit. PI-3 kinase activated via CD28 synergizes with enzymes activated via the TCR to enhance IL-2 production. Activated T cells express another B7 ligand, CTLA-4, which, in contrast to CD28, delivers a signal that terminates T-cell activation.

The B-cell antigen receptor signals in ways similar to the TCR. Surface Ig is associated with Igα and Igβ subunits, each of which has an ARAM motif. The src kinases lyn, blk and the ZAP-70 homologue syk associate with the sIg receptor. The B-cell-specific molecule CD19, which associates with the Ig receptor, contains, in its cytoplasmic tail, the YXXM motif. This mediates recruitment of PI-3 kinase.

The role of various enzymes and pathways is illustrated by several ID diseases, and by knockout mice. Disruption of $p56^{lck}$ leads to abnormal thymic maturation. Disruption of $p59^{fyn}$ affects only the function of peripheral T cells. ZAP-70 deficiency in humans results in CD8 deficiency and in deficient CD4+ T-cell function. In mice, ZAP-70 deficiency results in deficiency of both CD4 and CD8 cells. CD3γ and ϵ chain deficiency results in severely impaired T-cell receptor expression and variable impairment in T-cell function. NFAT abnormality leads to deficiency in the production of IL-2 and other cytokines.

6 ASSESSMENT OF PATIENTS SUSPECTED TO HAVE A PRIMARY IMMUNODEFICIENCY

6.1 Patient selection and identification

Early diagnosis and treatment of patients is vital. Many immunodeficiency diseases (ID), both primary and secondary, are treatable. Early treatment may prevent the otherwise inevitable devastating damage that can occur. Thus, whenever persistent or recurrent infections occur, which do not respond as expected to antibiotics, or which are caused by unusual or opportunistic infectious agents, primary or secondary ID must be considered. This is particularly important if family members have died in infancy or have similar susceptibility to infections. When such patients are encountered, studies should be carried out that permit identification of ID. Families of patients with ID should also be investigated.

Such patients can be divided into seven main groups: (a) infants from families known to have hereditary ID: prenatal diagnosis (see Section 8 below) is possible in many instances; (b) infants whose siblings have a possible or an established ID; (c) infants with syndromes or other diseases known to be associated with ID (see Section 10 below); (d) infants who fail to thrive, have unusually persistent infections with low virulence or opportunistic agents, unusual rashes, or persistent diarrhoea (see Section 9.2 below); (e) patients with recurrent or persistent infections that fail to respond as expected to antibiotic therapy; recurrent sinopulmonary infections are commonly the presenting problem (see Section 9.3 below), patients with chronic obstructive pulmonary disease should be investigated for ID; (f) patients with recurrent skin infections, abscesses, periodontitis, or unusual wound healing (see Section 12 below and Table 1); (g) patients with recurrent neisserial infections or with systemic lupus erythematosus (see Section 11 below and Table 2).

Normal neonates and young infants have higher lymphocyte counts than older children and adults. Lymphopenia in young infants should prompt investigation of ID. Normal or increased numbers of lymphocytes in babies and young children do not exclude the possibility of primary immune deficiency, not even the possibility of SCID or CID. These cells may be of maternal origin, even if graft vs. host (GvH) disease is not obvious. The presence of normal or increased IgM concentrations with the absence of other isotypes in young children is not limited to hyper-IgM syndrome and may be found in X-linked SCID and other forms of combined immune deficiency diseases. If a child for whom the possibility of a primary defect of cell-mediated immunity is suspected had been vaccinated post partum with BCG, tuberculostatic treatment should be started immediately.

The necessary screening for ID requires assessment of the patient's ability to develop and express B-cell, T-cell or combined T- and B-cell immunological functions. The biological amplification processes (complement, cytokines, etc.) and the basic effector mechanisms (phagocytosis and the inflammatory response) need to be investigated.

Evaluation should begin with enumeration of the crucial cell populations, T cells, B cells, granulocytes, monocytes; quantitative measurement of serum immunoglobulin concentrations of IgM, IgG, and in some instances IgA and IgE.

Immunological competence can be further assessed by quantification of specific antibody responses to ubiquitous antigens as well as to immunization with well-tolerated, commercially available antigens (e.g. tetanus and diphtheria toxoids, killed polio antigens, and haemophilus conjugates). Polysaccharide vaccines are obtainable; quantitative responses are difficult to evaluate because age, sex, ethnicity and race adjusted standards are not available. T-cell-mediated immunity can be determined by skin testing for delayed hypersensitivity with a battery of antigens, which in the aggregate yield positive responses in a high proportion of healthy individuals, but may be difficult to assess in infants

Table 1. Combined immunodeficiencies

Designation	Serum Ig	Circulating B cells	Circulating T cells	Presumed pathogenesis	Inheritance	Associated features
1. T-B + SCID*						
(a) X-linked (γc deficiency)	Decreased	Normal or increased	Markedly decreased	Mutations in γ chain of IL-2,4,7,9,15 receptors	XL	
(b) Autosomal recessive (Jak3 deficiency)	Decreased	Normal or increased	Markedly decreased	Mutation in Jak3	AR	
2. T-B−SCID						
(a) RAG 1/2 deficiency	Decreased	Markedly decreased	Markedly decreased	Mutation in RAG1/2 genes	AR	
(b) Adenosine deaminase (ADA) deficiency	Decreased	Progressive decrease	Progressive decrease	T-cell and B-cell defects from toxic metabolites (e.g. dATP, S-adenosyl homocysteine) due to enzyme deficiency	AR	
(c) Reticular dysgenesis	Decreased	Markedly decreased	Markedly decreased	Defective maturation of T and B cells and myeloid cells (stem cell defect)	AR	Granulocytopenia Thrombocytopenia
(d) Other	Decreased	Decreased	Decreased	Defective VDJ recombination	AR	
3. T + B−SCID						
(a) Omenn Syndrome	Decrease; Increased IgE	Normal or Decreased	Present; Restricted heterogeneity	Missense mutations in RAG1/2 genes	AR	Erythrodermia; Eosinophilia; Hepatosplenomegaly
(b) IL-2Rα Deficiency	Normal	Normal	Decreased	Mutation in IL-2Rα gene	AR	Lymphadenopathy Hepatosplenomegaly
4. X-linked hyper IgM	IgM & IgD increased or normal; other isotypes decreased	IgM & IgD bearing cells present others absent	Normal	Mutations in CD40 ligand gene	XL	Neutropenia Thrombocytopenia Haemolytic anaemia Gastrointestinal & liver involvement
5. Purine nucleoside phosphorylase (PNP) deficiency	Normal or decreased	Normal	Progressive decrease	T-cell defect from toxic metabolites (e.g. dGTP) due to enzyme deficiency	AR	Autoimmune haemolytic anaemia: neurological symptoms
6. MHC class II deficiency	Normal or decreased	Normal	Normal, decreased CD4 numbers	Mutation in transcription factors (CIITA or RFX5, RFXAP, RFXANK genes) for MHC class II molecules	AR	
7. CD3γ or CD3ϵ deficiency	Normal	Normal	Normal	Defective transcription of CD3γ or CD3ϵ chain	AR	
8. ZAP-70 deficiency	Normal	Normal	Decreased CD8, normal CD4	Mutations in Zap-70 kinase gene	AR	
9. TAP-2 deficiency	Normal	Normal	Decreased CD8, normal CD4	Mutations in TAP-2 gene	AR	MHC class I deficiency

* Atypical cases of γc or Jak3 deficiency may present with T cells.

and younger children because such immunity has not yet been acquired. T-cell immunity can also be evaluated by *in vitro* responses of peripheral blood lymphocytes to phytomitogens, common antigens, and/or anti-CD3.

In those patients with relevant symptoms (see Section 11 below) total haemolytic complement and complement components of both the classical and alternative activation pathways should be measured immunochemically and functionally.

Patients suspected of having chronic granulomatous disease should have phagocytic function evaluated by: (a) semiquantitative nitro blue tetrazolium dye reduction after exposure of patient's peripheral blood cells to a phagocytic stimuli; (b) measurement directly of O_2- radical formation after stimulation of patient's blood cells using PMA or dichlorfluorine; and quantitatively measuring O_2- production; (c) by measuring the phagocytic response using chemiluminescence following similar stimulation; (d) by quantifying the capacity of patient's cells to ingest and kill catalase-positive microorganisms such as staphylococci or paracolobacilli. The integrity of the inflammatory response can be tested by Rebuck skin window techniques. *In vitro* analysis

Table 2. Predominantly antibody deficiencies

Associated designation	Serum Ig features	Circulating B cells	Presumed pathogenesis	Inheritance	Associated features
1. X-linked agammaglobulinaemia	All isotypes decreased	Profoundly decreased	Mutations in *btk*	XL	–
2. Autosomal recessive agammaglobulinaemia	All isotypes decreased	Profoundly decreased	Mutations in μ or $\lambda 5$ genes; others	AR	–
3. Ig heavy-chain gene deletions	IgG1 or IgG2, IgG4 absent and in some cases IgE and IgA1 or IgA2 absent	Normal or decreased	Chromosomal deletion at 14q32	AR	–
4. κ Chain deficiency mutations at AR	Ig(K) decreased: antibody response normal or decreased	Normal or decreased κ-bearing cells	Point mutations at chromosome 2p11 in some patients	AR	–
5. Selective Ig deficiency				Unknown	–
(a) IgG subclass deficiency	Decrease in one or more IgG isotypes	Normal or immature	Defects of isotype differentiation		
(b) IgA deficiency	Decrease in IgA1 and IgA2	Normal or decreased sIgA +	Failure of terminal differentiation in IgA + B cells	Variable	Autoimmune and allergic disorders
6. Antibody deficiency with normal or elevated Igs	Normal	Normal	Unknown	Unknown	–
7. Common variable immunodeficiency	Decrease in IgG and usually IgA, ± IgM	Normal or decreased	Variable; undetermined	Variable	See text Section 9.3.6
8. Non X-linked hyper IgM syndrome	IgM and IgD increased or normal other isotypes decreased	IgM and IgD bearing cells present others absent	Unknown		Neutropenia Thrombocytopenia Haemolytic anaemia Gastrointestinal and liver involvement
9. Transient hypogammaglobulinaemia of infancy	IgG and IgA decreased	Normal	Differentiation defect: delayed maturation of helper function	Unknown	Frequent in families with other IDs

of the inflammatory response can be studied by measurement of chemotaxis, chemokinesis, and the capacity to produce and release selected inflammatory cytokines. Expression of adhesion molecules (e.g. CD18) should be assessed.

Diagnosis of several IDs can be performed at the level of the respective genes responsible for the immunodeficiency. Analysis of the gene at the molecular level as well as demonstration of the respective gene products may be performed in specialized laboratories.

6.2 Immunoglobulins and antibodies

6.2.1 Measurement of immunoglobulin concentration. Serum immunoglobulins are commonly measured by radial immunodiffusion or automated immunoturbidometric methods. Other techniques such as radioimmunoassay and ELISA are also available and useful for IgE and IgD measurements. Quality assessment (QA) is widely available throughout the world to ensure reliability. Electrophoresis and immunoelectrophoresis are not satisfactory techniques for the quantification of immunoglobulins. Immunoelectrophoresis and immunofixation are, however, useful in the detection of M-components. Immunoglobulin can also be measured in body secretions, e.g. saliva, tears and milk, but this is rarely indicated. Monomers of IgM (usually a pentamer) are present in serum of some ID patients such as CVID and hyper-IgM and may give spuriously high IgM levels. The subclasses of IgG can be measured by simple radial diffusion or ELISA methods. Although standards and normal values for IgG subclasses are now in use, the ranges in normal children are very wide and do not take into consideration genetic and geographical variations. IgG subclass determination is of limited value in assessing patients with clinical immunodeficiency, because functional antibody deficiency may be present despite normal IgG subclass levels, and conversely deficient levels of a single subclass of IgG may be found in individuals who have effective specific antibody production and are clinically normal. Methods for IgA subclass determinations are not yet readily available and their measurement is not yet of value. Serum immunoglobulin concentrations vary with age and environment; thus appropriate regional and ethnic age-related and sex-related norms must be used.

Concentrations of immunoglobulins cannot be used as the sole criteria for the diagnosis of primary ID. Diminished immunoglobulin levels may be due to loss as well as decreased synthesis. An indirect indication of loss may be obtained by measuring serum albumin, which is usually lost concomitantly (e.g. through the gastrointestinal or renal tracts). Limited heterogeneity of immunoglobulins and abnormal kappa–lambda light-chain ratios have been observed in ID syndromes.

6.2.2 Assessment of antibody formation following immunization. Antibody-mediated immunity (humoral immunity) may be assessed by antibody responses to those antigens to which the population is commonly exposed, or following active immunization. Protein or polysaccharide antigens may be used; the latter are particularly relevant in patients with sinopulmonary infections. It is important that sensitive and reproducible assays are used, such as ELISA. Normal ranges of IgG antibodies in children following immunization are available but need careful interpretation.

Live vaccines (e.g. BCG and vaccines for poliomyelitis, measles, rubella and mumps) should never be given, even to family members, when primary ID is suspected. BCG is contraindicated in patients with T-cell or phagocytic cell IDs. Live viral vaccines, including polio, measles, mumps and rubella vaccination must not be given in patients with T-cell ID (as defined by an absence of antigen-specific response, e.g. TT) and XLA or other severe B-cell deficiencies. Live vectors are also contraindicated in patients with ID.

The following tests are recommended:

1. 'Natural' antibodies: A and B isohaemagglutinins are sometimes used as measures of IgM antibodies.
2. IgG antibody responses to common immunizations.

(a) In non-immunized children, commercial diphtheria/tetanus (DT) or Hib-conjugate vaccines may be given in recommended doses. Blood is taken 3 weeks after the last immunization and IgG antibodies determined, usually by ELISA. A Schick test may be performed for diptheria antibodies. Three doses of killed poliomyelitis vaccine (1.0 ml intramuscularly, at intervals of 2 weeks) can also be used; blood is taken 2 weeks after the last injection and antibody determined, usually by virus neutralization.

(b) In patients who have been immunized with diphtheria/tetanus (DT) or diphtheria/pertussis/tetanus (DPT) vaccine, IgG antibodies are measured; if low, one booster injection is given, followed by measurement of antibodies and/or a Schick test. The widespread routine use of *Haemophilus influenzae* type b (Hib) conjugate vaccine makes measurement of IgG antibodies to Hib valuable if this immunization has been completed.

3. Additional active immunizations that may be recommended.

(a) Bacteriophage ϕX 174, a bacterial virus that is not infective for humans, has been shown to be a potent, safe and useful antigen; it allows measurement of antigen clearance and primary and secondary immune responses[a].

(b) To measure IgG antibody responses purely to carbohydrate antigens[b], pneumococcal or meningococcal polysaccharides, or Haemophilus b, polysaccharide free of carrier proteins can be used as well as typhoid-Vi antigen. Blood is drawn after 3 weeks and IgG antibody is determined by ELISA. These and other pure polysaccharide antigens are not useful in infants under 2 years of age. Interpretation of results in children under 5-years of age is difficult, as the age at which these responses develop ranges from 2 to 4 years of age.

(c) Other useful antigens to measure primary response include: (i) tick-borne encephalitis (killed) vaccine[c]; (ii) hepatitis A vaccine[d]. Hepatitis B is not a reliable antigen for testing immune competence because of the high frequency of nonresponders in the population, particularly in patients over 40 years of age.

6.2.3 B lymphocytes. B lymphocytes are counted by detection of the membrane-bound CD antigens CD19 and CD20. This can be done either by using a flow cytometer and fluorescent-labelled monoclonal antibodies to B-cell antigens or by immunohistological techniques on whole blood films. Monocytes can be distinguished from B lymphocytes by gating on a flow cytometer, by peroxidase or esterase staining on films, ingestion of IgG-coated latex particles or by monoclonal antibodies specific for monocytes, such as to CD14.

Pre-B cells may be identified among bone marrow cells with purified fluorochrome-labelled antibodies to detect cytoplasmic μ heavy chains in CD19 + cells.

6.3 Cell-mediated immunity (CMI)

A number of tests are commonly employed for assessing CMI, including: delayed-type skin reactions; enumeration of T cells and T-cell subsets; *in vitro* tests of T-cell function.

6.3.1 Skin testing. Delayed cutaneous hypersensitivity (DCH) is a localized immunological skin response: the prototype is the tuberculin skin test. Because DCH is dependent on functional thymus-derived lymphocytes (T lymphocytes), DCH may be used in screening for T-cell-mediated immunodeficiency. Antigens generally used are: mumps, trichophyton, purified protein derivative (PPD), candida or monilia, tetanus or diphtheria toxoids. To ascertain defective CMI several antigens must be used. All skin tests are performed by intradermal injection of 0.1 ml of antigen. Results should be read in 48–72 h for the maximal diameter of induration, which indicates intact CMI. Erythema is not an indication of DCH.

1. Tuberculin: 0.1 ml containing 2–10 international units (IU) of Tween-stabilized soluble PPD.
2. Candida or monilia[e]: initially test at 1:100 dilution. If no reaction, test at 1:10 dilution.
3. Trichophyton[f]: use at 1:30 dilution.
4. Mumps[g]: use undiluted; read at 6–8 h for early Arthus reaction (antibody mediated) and then at 48 h for DCH.
5. Tetanus and diphtheria fluid toxoids[h]: use at 1:100 dilution.

A positive DCH is informative while a negative DCH test may be difficult to interpret. This is because DCH is influenced by age, steroid therapy, severe illness and recent immunization.

We do not recommend the use of dinitrochlorobenzene (DNCB) for skin testing; it is mutagenic and can cause necrosis. We also do not recommend the use of any multitest system for assessing CMI.

6.3.2 T lymphocytes. Because of the reliance on the phenotypic designation of T-cell subsets in evaluating patients with ID, it is essential to understand the normal differentiation and functions of these cells (see Section 2).

T cells can be enumerated by immunofluorescence with the use of monoclonal antibodies to CD3. Monoclonals to CD3 enumerate NK as well as T cells. Flow cytometry techniques are more reliable, reproducible and sensitive than visual microscopic enumeration; if a flow cytometer is not available, immunohistological techniques using either enzymatic or immunofluorescent-labelled antibodies can be used. CD4 and CD8 monoclonal antibodies recognize important subsets of T cells, although monoclonal antibodies to CD8 enumerate NK as well as T cells. CD4 cells recognize antigen in association with the class II MHC (HLA-D) molecules, and CD8 cells recognize antigens in association with class I MHC (HLA-A, HLA-B and HLA-C) molecules. Antigen-specific T-cell responses are MHC restricted. Abnormalities in the number of CD4 or CD8 cells can be associated with abnormalities in cognate as well as regulatory functions of T cells and may lead to immuno-incompetence or auto-immunity.

In suspected cases of hyper-IgM immunodeficiency, T cells activated by PMA and ionomycin should be analysed for expression of the CD40 ligand.

6.3.3 In vitro *stimulation of lymphocytes.* Lymphocytes can be activated in vitro by (a) mitogens such as phytohaemagglutinin (PHA) pokeweed mitogen (PWM) or concanavalin A (Con A); (b) antigens such as PPD, candida, streptokinase, tetanus and diphtheria, if the patient has had a prior encounter with the antigen or with superantigens such as toxic shock syndrome toxin (TSST); (c) allogeneic cells; and (d) antibodies to T-cell surface molecules involved in signal transduction such as to CD3, CD2, CD28 and CD43.

T-lymphocyte activation can be assessed directly by (a) expression of activation antigens; (b) measuring blastogenesis and/or proliferation of cells; and (c) release of mediators.

A rapid result can be detected by detection of activation markers. Activated T cells express CD69, IL-2 receptors alpha (CD25), transferrin receptors (CD71) and MHC class II molecules not present or present in low numbers on resting T cells. T-cell populations to be assessed for their capacity to express these receptors are stimulated with a soluble lectin such as PHA, and examined 1–2 days later by direct or indirect immunofluorescence using monoclonal antibodies to CD25, or transferrin CD71 or MHC class II molecules and a flow cytometer.

The blastogenic response is assayed after 3–7 days depending on the nature of the stimulant, by ^{3}H-or ^{14}C-labelled thymidine incorporation for 16–24 h. This is followed by DNA extraction or cell precipitation on filter paper and subsequent liquid scintillation counting. Control values of unstimulated cultures vary from person to person and from day to day. Data on unstimulated and stimulated cultures should always be given. Soluble PHA or Con A require the presence of monocytes for stimulation of T cells; under certain conditions, however, such as when bound to particulate matter, they may also stimulate B cells. PWM stimulates a response to both T and B cells, although T cells must be present for the B cells to be stimulated. The mixed lymphocyte reaction (MLC) results from T-cell reactivity to MHC antigens displayed on B cells and monocytes. It should be noted that when normal irradiated or mitomycin C-treated lymphocytes are the stimulators of an MLC, the normal T cells in the culture may secrete factors that induce blastogenesis in the patient's lymphocytes. Therefore it is preferable to use B-cell lines or T-cell-depleted normal cells as the stimulators.

Activated T-cells and monocytes synthesize and secrete interleukins-2,4,5 and 6, interferon-γ and other cytokines. The supernatants of peripheral blood mononuclear cells stimulated by soluble PHA can be assessed for IL-2 by an ELISA technique or by determining their capacity to stimulate ^{3}H-thymidine uptake by mouse IL-2-dependent cultured T-cell lines (e.g. CTLL2). The bioassay should be confirmed with blocking antibodies to IL-2, because other cytokines (e.g. IL-15) also activate this system. Specific *in vitro* systems also exist to assay other cytokines. It may soon be possible to measure synthesis of intracellular cytokines reliably and quantitatively.

6.4 NK cells

Monoclonal antibodies against CDl6, CD56 and CD57, even though they are not lineage specific, may be useful for the detection and enumeration of NK cells. NK functional activity can be assessed by a cytotoxicity assay against cell line such as K562. This is important in the diagnosis of SCID, Chediak–Higashi syndrome and rare cases of isolated NK cell deficiencies.

7 OTHER TESTS

Examination of blood is essential, and biopsies of bone marrow, rectum and intestine, skin and lymphoid tissue may also be warranted for the diagnosis or classification of ID. In addition, postmortem examination may permit diagnosis of familial defects, important for genetic counselling and for understanding the pathogenesis of ID.

7.1 Blood

A total lymphocyte count is essential in the diagnosis of primary ID. Most patients with SCID and thymic hypoplasia have persistently low total lymphocyte counts (less than 1.5×10^{9}/l or l500/mm^{3}). Lymphopenia can also be secondary to viral infections, malnutrition, cell loss, autoimmune diseases and myelophthisis as in haematopoietic malignancy. Normal lymphocyte counts do not exclude the diagnosis of SCID. Lymphocyte counts are variable in other forms of ID. Patients with reticular dysgenesis have pancytopenia. Thrombocytopenia and small platelets in a male infant suggest the Wiskott–Aldrich syndrome. Some patients with ID are anaemic; this may include a Coombs' positive haemolytic anaemia.

7.2 Bone marrow

Bone marrow aspiration or biopsy is important for exclusion of other diseases, for identification of plasma cells and pre-B cells and for diagnosis of obscure infections.

7.3 Lymph nodes

Lymph node biopsy is not necessary for the diagnosis of most ID but may be helpful. Rapidly enlarging lymph nodes should be biopsied; infection, malignancy or follicular hyperplasia may be the cause. Lymph node biopsies are potentially hazardous in SCID; they heal poorly and may produce a portal of entry for infection.

7.4 Rectal and intestinal biopsy

Examination of rectal tissue for plasma cells and lymphoid cells by histological and immunohistological methods may be informative in patients with common variable ID and selective IgA deficiency. Lymphoid cells are found in rectal and intestinal biopsies in normal infants more than 15–20 days old. Jejunal biopsy may show villous atrophy and may demonstrate *Giardia lamblia* and cryptosporidial infections.

7.5 Skin biopsy

Biopsy of skin is useful to establish a diagnosis of GvH reaction in patients with ID after blood transfusion, bone marrow and fetal tissue transplantation or from maternal/fetal transfer of lymphocytes *in utero*.

7.6 Thymus

Thymic biopsy should be performed only by skilled surgeons. It should be performed only when thymoma is suspected.

7.7 Chimerism

Chimerism (the occurrence in one individual of two genetically different cell lines) when observed in ID can be congenital or acquired. The former is due to intrauterine implantation of maternal cells; the latter can occur after blood transfusion, bone marrow transplantation or fetal tissue implants. The presence and origin of lymphoid chimeric cells can be ascertained by karyotype, human leucocyte (HLA) or other antigenic typing, and analysis of highly polymorphic markers.

7.8 Special studies

Adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) levels should be determined in all patients with possible SCID and T-cell deficiency. Serum alpha fetoprotein levels may be of value in separating patients with ataxia-telangiectasia (A-T) from those with other neurological disorders; it is increased (40–2000 μg/l) in at least 95% of patients with A-T. In patients with SCID, blood mononuclear cells should be examined for the presence of class II histocompatibility molecules to rule out the diagnosis of MHC class II deficiency. Cytogenic analysis is useful in diagnosis of A-T and other chromosomal breakage syndrome. Molecular cytogenetic studies are helpful in the diagnosis of the DiGeorge anomaly and should be performed. This may be informative in other conditions (see Section 10).

7.9 Studies for infectious agents

The diagnosis of infection in ID is complex and beyond the scope of this report. In patients with ID, diagnoses of viral infection by antibody determinations are of little or no value because the patients have defective antibody formation. Direct viral isolation and/or identification of the viral genome (e.g. by PCR) are necessary to prove infection. In the presence of CNS symptoms, CSF cultures as well as PCR tests are important and brain biopsy may be required. HIV can be detected in peripheral blood lymphocytes and plasma by PCR analysis. Bronchial lavage may be useful for the diagnosis of *Pneumocystis carinii* and other pulmonary pathogens.

8 GENETICS CARRIER DETECTION AND PRENATAL DIAGNOSIS

Inheritance patterns are known for most of the primary immunodeficiency diseases and are given in Tables 1, 2, 3, 4, and 5. The known chromosomal map locations of several immunodeficiencies are given in Table 6. Table 5 contains the presently known chromosomal map locations of the complement genes. These recent advances in the precise mapping of the various ID and the availability of highly polymorphic markers often makes carrier detection and prenatal diagnosis possible (Table 7).

Table 3. Other well-defined immunodeficiency syndromes

Designation	Serum Ig and antibodies	Circulating B cells	Circulating T cells	Genetic defect	Inheritance	Associated features
1. Wiskott–Aldrich syndrome	Decreased IgM: antibody to polysaccharides particularly decreased; often increased IgA and IgE	Normal	Progressive decrease	Mutations in WASp gene; cytoskeletal defect affecting haematopoietic stem cell derivatives	XL	Thrombocytopenia; small defective platelets; eczema; lymphomas; autoimmune disease
2. Ataxia-telangiectasia	Often decreased IgA, IgE and IgG subclasses; increased IgM monomers; antibodies variably decreased	Normal	Decreased	Mutation in A-T gene (*ATM*); disorder of cell cycle checkpoint pathway leading to chromosomal instability	AR	Ataxia; telangiectasia; increased alpha fetoprotein; lympho-reticular and other malignancies; increased X-ray sensitivity
3. Nijmegen breakage syndrome	as in no. 2	Normal	Decreased	Defect in *NBS1* (*Nibrin*); disorder of cell cycle check point and DNA double strand break repair	AR	Microcephaly lymphomas; ionizing radiation sensitivity; chromosomal instability
4. DiGeorge anomaly	Normal or decreased	Normal	Decreased or normal	Contiguous gene defect in 90% affecting thymic development	De novo defect or AD	Hypoparathyroidism: conotruncal malformation; abnormal facies; partial monosomy of 22q11-pter or 10p in some patients
5. Imunodeficiency with albinism						
(a) Chediak–Higashi syndrome	Normal	Normal	Normal	Defect in *Lyst*	AR	Albinism; acute phase reaction; low NK and CTL activities; giant lysosomes
(b) Griscelli syndrome	Normal	Normal	Normal	Defect in *myosin 5a*	AR	Albinism; acute phase reaction; low NK and CTL activities; progressive encephalopathy in severe cases
6. X-linked proliferative syndrome	Normal or rarely hypogamma-globulinaemia	Normal or reduced	Normal	Defect in *SAP*	XL	Clinical and immunological manifestations induced by EBV infection; hepatitis; aplastic anaemia; lymphomas

Table 4. Complement deficiencies

Deficiency	Inheritance	Chromosomal location	Concommitant symptom
C1q	AR	1	SLE–like syndrome, rheumatoid disease, infection
C1r*	AR	12	SLE–like syndrome, rheumatoid disease, infection
C4	AR	6	SLE–like syndrome, rheumatoid disease, infection
C2**	AR	6	SLE–like syndrome, vasculitis, polymyositis, pyogenic infection
C3	AR	19	Recurrent pyogenic infections
C5	AR	9	Neisserial infection, SLE
C6	AR	5	Neisserial infection, SLE
C7	AR	5	Neisserial infection, SLE, vasculitis
C8α***	AR	1	Neisserial infection, SLE
C8β	AR	1	Neisserial infection, SLE
C9	AR	5	Neisserial infection
C1 inhibitor	AD	11	Hereditary angioedema
Factor I	AR	4	Recurrent pyogenic infections
Factor H	AR	1	Recurrent pyogenic infections
Factor D	AR	19	Neisserial infection
Properdin	XL	X	Neisserial infection

* C1r deficiency in most cases is associated with C1s deficiency. The gene for C1s also maps to chromosome 12 pter.

** Type 1 C2 deficiency is in linkage disequilibrium with HLA-A25, B18 and -DR2 and complotype, SO42 (slow variant of Factor B, absent C2, type 4 C4A, type 2 C4B) and is common in Caucasians. It results from a 28-bp deletion in the C2 gene; C2 is synthesized but not secreted. Type 2 C2 deficiency is very rare and involves gene defects other than that found in Type 1 C2 deficiency and a failure of C2 synthesis.

*** C8α deficiency is always associated with C8γ deficiency. The gene encoding C8γ maps to chromosome 9 and is normal but C8γ covalently binds to C8α.

Carrier detection can be accomplished in several of these diseases. Where the location of the gene has been reasonably established (see Table 6), polymorphic markers may, in informative families, identify carriers with reasonable certainty. In those instances where a specific enzyme or complement component defect is present, heterozygote carriers can be ascertained from reduced levels of the enzyme or component in question. In some X-linked diseases, preferential selection against cells carrying the abnormal X chromosome during cell proliferation and differentiation of affected cell lineages permits determination of the carrier status. Preferential selection does not occur in carriers of the X-linked hyper-IgM syndrome or X-linked chronic granulomatous disease.

At the present time, prenatal diagnosis can be made by obtaining fetal blood samples, amnion cells or chorionic villus biopsy. In some ID polymorphic markers can be used to establish diagnosis prenatally. The absence of B or T lymphocytes from umbilical cord blood can be used for the prenatal diagnosis of X-linked agammaglobulinaemia and SCID, respectively. However, whenever possible, prenatal diagnosis should be accomplished by molecular tests. Chronic villi are preferable to fetal blood samples to ascertain adenosine deaminase or purine nucleoside phosphorylase deficiency. Absence of cell membrane components such as MHC class II molecules and CD18 on fetal blood cells can identify MHC Class II deficiency and the leucocyte adhesion defect 1.

9 PRIMARY SPECIFIC IMMUNODEFICIENCY

9.1 Introduction

Nomenclature and characteristics of currently recognized primary immunodeficiency diseases are given in Tables 1, 2, 3 and 7. The columns provide the points below.

9.1.1 Designated nomenclature. Nomenclature that defines the presumed cause or the most characteristic expression of the disease is generally used. Eponyms have been avoided because original descriptions from which they were derived preceded modern immunological techniques and, as a result, may be misleading. Precise nomenclature and standardized diagnostic criteria are crucial for case documentation, comparison and compilation of registries.

9.1.2 Serum immunoglobulin levels. Defective antibody formation is the most common abnormality in the majority of primary immunodeficiency diseases. It is generally reflected by decreased total serum Ig. Thus serum antibody and serum Ig concentrations are combined under a single heading.

9.1.3 Circulating B and T lymphocytes. Enumeration and characterization of circulating lymphocytes is essential for the diagnosis. Methods for T- and B-lymphocyte enumeration and differentiation markers are given in Sections 6.2.3 and 6.3.2 and for function analysis in Sections 6.3.1 and 6.3.3. Skin tests for delayed hypersensitivity (CMI) generally reflect T-lymphocyte numbers and *in vitro* functional assays, they are thus omitted from the listed characteristics.

9.1.4 Presumed pathogenesis. Many primary immunodeficiency diseases result from impeded B or T lymphocyte development and differentiation. The normal ontogeny is described in Section 2, and is schematically shown in Fig. 1. The probable location of the arrest in development or differentiation is indicated where possible. In the few instances where the defect can be more precisely identified, greater details are given.

9.1.5 Inheritance. Many of the primary immunodeficiency diseases are inherited. The inheritance of those that have been well defined is noted. Approximate chromosomal gene map location is given in Table 6.

Table 5. Congenital defects of phagocytic number and/or function

Disease	Affected cells	Functional defects	Inheritance	Features
Severe congenital neutropenia	N	–	AR	
Cyclic neutropenia	Mainly N	–	AR	Oscillations of reticulocytes, platelets and other leucocytes
Leucocyte adhesion defect 1 [deficiency of beta chain (CD18) of LFA-1, Mac 1, p150,95]	N + M + l + NK	Chemotaxis, adherence, endocytosis	AR	Delayed cord separation, chronic skin ulcers, periodontitis, leucocytosis, defective T + NK-cell cytotoxicity
Leucocyte adhesion defect 2 (failure to convert GDP mannose to fucose)	Mainly N + M	Chemotaxis, rolling	AR	Delayed wound healing, chronic skin ulcers, periodontitis, mental retardation, leucocytosis, Bombay blood group
Specific granule deficiency	N	Chemotaxis	AR	N with bi-lobed nuclei
Shwachman syndrome	N	Chemotaxis	AR	Anaemia, thrombocytopenia, pancreatic insufficiency, chondro-dysplasia, hypogammaglobulinaemia
Chronic granulomatous disease				
(a) X-linked CGD (deficiency of 91 kDa chain of cytochrome b)	N + M	Killing (faulty production of superoxide metabolites)	XL	McLeod phenotype*
(b) Autosomal recessive (deficiencies of 22 kDa chain of cytochrome b or of p47 or p67 cytosol factors)	N + M	Killing — as above	AR	–
Neutrophil G6 PD deficiency	N + M	Killing	XL	Anaemia
Myeloperoxidase deficiency	N	Killing	AR	This deficiency may be found in normal people
Leucocyte mycobactericidal defect (a) IFN-γ receptor 1 deficiency (b) IFN-γ receptor 2 deficiency (c) Interleukin-12 receptor deficiency (d) Interleukin-12 deficiency	N + M + L + NK	Killing	AR	Extreme susceptibility to mycobacteria and salmonella

N = neutrophils; M = monocytes/macrophages; L = lymphocytes; NK = natural killer cells.

* Some patients have deletions in the short arm of the X chromosome; in these patients additional features including McLeod phenotype, retinitis pigmentosa and Duchenne muscular dystrophy may be found.

9.1.6 Associated features. Commonly associated, characteristic and often diagnostic nonimmunological features for some of the primary immunodeficiency diseases are listed. Additional conditions that have been associated with immunodeficiency are described in Section 10.

9.2 Combined immunodeficiencies (CID)

This group of diseases (Table 1) is characterized clinically and immunologically by defects in both T and B lymphocytes. Criteria for diagnosis generally include presentation in infancy with severe, potentially lethal infections, profound abnormalities of CMI and antibody deficiency, and lymphopenia, particularly of T lymphocytes. The clinical presentation usually includes failure to thrive and unusually persistent infections with low virulence opportunistic organisms (for example, Candida, *Pneumocystis carinii*, cytomegalovirus). These findings require differentiation from infants with acquired immunodeficiency syndrome (AIDS). HIV studies should include viral isolation or PCR studies for viral genome. SCID is further distinguished on the basis of pathogenesis where known (e.g. enzyme defects), mode of inheritance and level of faulty differentiation.

9.2.1 Severe combined immunodeficiency (SCID). Patients with SCID can be divided into two large groups: (i) those who lack both T and B lymphocytes (T-B−SCID), and (ii) those who have normal to increased B cells, and lack of T lymphocytes (T-B+ SCID). X-linked, T-B+ SCID is the most common form of SCID, and is due to mutations in the γc chain shared by the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15. Autosomal recessive, T-B+ SCID is due to mutations of the intracellular kinase, Jak3, that binds to γc. Some patients with SCID have symptoms similar to GvH in the neonatal period. This has been termed 'Omenn's Syndrome'; the disease is not, however, due to engraftment of maternal cells. The genetic and molecular bases of several forms of SCID have been determined and are listed in Table 3 and described in Section 9.4. Bone marrow transplantation is the treatment of choice for SCID, with excellent results.

9.2.1.1 RAG-1/-2 deficiency. Many patients with T-B−SCID

Table 6. Chromosome map location of IDs listed in Tables*

1. X-linked severe combined immunodeficiency	Xq13.1–13.3
2. X-linked agammaglobulinaemia	Xq21.3–22
3. X-linked immunodeficiency with increased IgM	Xq26–27
4. Wiskott–Aldrich syndrome	Xp11.22–11.3
5. X-linked chronic granulomatous disease	Xp21.1
6. X–linked lymphoproliferative syndrome	Xq26
7. Adenosine deaminase deficiency	20q13-ter
8. Purine nucleoside phosphorylase deficiency	14q13.1
9. ZAP-70 deficiency	2q12
10. Jak3 deficiency	19p13.1
11. RAG-1/RAG-2	11p12–13
12. Kappa chain deficiency	2p11
13. Ig heavy chain deletion	14q32.3
14. Ataxia-telangiectasia	11q23.1
15. Autosomal recessive chronic granulomatous disease	
p22 phox	16q24
p47 phox	7q11.23
p67 phox	1q25
16. Leucocyte adhesion deficiency 1	21q22.3
17. IFN-γR α chain deficiency	6q16–21
18. Chediak–Higashi syndrome	1q4.3
19. TAP-2	6p21.3
20. CIITA	16p13.1–2
21. RFX5	1q21
22. RFXAP	13q
23. RFXANK	19p12

*The complement gene map locations (and hence the deficiencies thereof) are given in Table 4.

have mutations in RAG-1 or RAG-2 genes. These infants have very few B or T cells but may have normal or increased numbers of NK cells. The defects are inherited as an autosomal recessive.

9.2.1.2 Adenosine deaminase (ADA) deficiency. There is a group of distinctive patients whose SCID results from defects in the enzyme ADA. This group of phenotypically similar genetic defects include various mutations within the gene encoding ADA on chromosome 20q13-ter. In the absence of ADA, toxic metabolites of the purine pathway (dATP) and the methylation pathway (S-adenosyl homocysteine) accumulate within the cell and impair proliferation; as a result both T- and B-lymphocyte functions are defective. Inheritance of the defect is autosomal recessive. Rare patients with certain mutations in *ADA* that result in a mild phenotype may have late onset of ID.

9.2.1.3 Reticular dysgenesis. This rare hereditary autosomal recessive disease is generally lethal shortly after birth. It results from failure in the maturation of both lymphoid and myeloid precursors, and is characterized not only by striking lymphopenia, but also by severe granulocytopenia and thrombocytopenia, and overwhelming infections with early death. Engraftment of maternal T cells in SCID infants may occasionally mimic reticular dysgenesis.

9.2.2 Immunoglobulin deficiency with increased IgM (the hyper-IgM syndrome). This syndrome apparently represents a group of distinct entities with similar clinical (and phenotypic) expression. Approximately 70% of the cases are X-linked in inheritance; others have been autosomal recessive. Diagnostic criteria include impeded antibody formation. Patients may have an intact IgM antibody response. There is no switch to IgG antibody formation. Thus serum IgM (and sometimes IgD) levels are elevated while IgG and IgA levels are diminished. Circulating B lymphocytes bear only IgM and IgD. The defect is a failure of isotype switch but there is no defect in the switch regions of B lymphocytes. Most patients have recurrent or persistent neutropenia and thrombocytopenia. Defects in CMI have been noted in some patients.

In the X-linked form, the genetic defect has been identified in mutation of the gene for the CD40 ligand, which is expressed on activated T lymphocytes. The interaction of the CD40 ligand with CD40 on B lymphocytes is requisite for productive isotype switching. The gene for the CD40 ligand maps to Xq26, where the hyper-IgM syndrome had previously been mapped. The CD40 ligand is a type 2 glycoprotein that belongs to the same gene family as tumour necrosis factor. In most cases no CD40 ligand is expressed on the T cells of these patients. In others a mutant nonfunctional protein is expressed and these patients may have a less severe phenotype.

9.2.3 Purine nucleoside phosphorylase (PNP) deficiency. This autosomal recessive disease results from defects in the gene

Table 7. Prenatal diagnosis

Diseases	Direct/indirect gene analysis	Findings in fetal cord blood or amnion cells
X-linked agammaglobulinaemia	+	Absence of B cells
X-linked severe combined immunodeficiency	+	Absence of T cells
Autosomal recessive severe combined immunodeficiency	–	Absence of T cells (and B cells)
Wiskott–Aldrich syndrome	+	'Bald' lymphocytes by scanning EM
Ataxia-telangiectasia	+	Radiosensitivity
MHC class II deficiency	–	Absence of MHC class II molecules on cell membranes
Leucocyte adhesion deficiency	(*)	Absence of CD18 on phagocytes
X-linked chronic granulomatous disease	+	Abnormal oxygen radical production
Autosomal recessive chronic granulomatous disease	(*)	Abnormal oxygen radical production
Adenosine deaminase deficiency	+	Decreased ADA in red blood cells
Purine nucleoside phosphorylase deficiency	+	Decreased PNP in red blood cells

*Potentially possible, but not yet well established.

encoding the enzyme PNP located on chromosome 14. In the absence of PNP, toxic metabolites, in this case dGTP, accumulate within the cell and impair proliferation. T lymphocytes are particularly sensitive to the accumulation of dGTP and they are affected to a greater degree than B lymphocytes. There are thus immunological differences between ADA and PNP deficiency.

9.2.4 MHC class II deficiency. The disease is due to a defect in proteins that promote transcription of class II molecules. The disease is heterogeneous and four complementation groups are presently known. Complementation group A results from mutations in the gene encoding class II transcription activation (CIITA). Complementation group C results from mutation in the genes for the heterodimer RFX5, whereas mutations of p36 (that binds to RFX5) account for complementation group D. In the absence of class II MHC molecules, cognitive functions, particularly those involving CD4+ T lymphocytes, are impaired. Circulating lymphocyte numbers are normal, but CD4+ T cells are decreased. Antibody synthesis and serum immunoglobulins are decreased and CMI is defective. Several of these children have been recipients of successful bone marrow transplants. An unusual phenotype with residual expression of HLA-DRα and β chains is associated with a normal number of circulating CD4+ lymphocytes, and a more benign clinical course.

9.2.5 CD3 deficiency. The phenotype of CD3 deficiency may be variable, even within a family, due to variable expression of CD3 on the T-cell membrane. Deficiencies or abnormalities of CD3 γ and ϵ have been described.

9.2.6 ZAP-70 deficiency. This rare deficiency is inherited as an autosomal recessive trait and is due to mutations in the gene for ZAP-70 (see Fig. 4), a tyrosine kinase involved in TCR signalling. CD4+ T cells are present in normal or elevated numbers but are not functional. Some of these children have been recipients of successful bone marrow transplants.

9.2.7 TAP 2 peptide transporter deficiency. In one family, an immunodeficiency characterized by reduced HLA class I molecules expression, low CD8 T-cell counts and defective cytotoxicities have been found in association with mutation in the gene encoding the peptide transporter chain TAP 2. The latter is necessary for the transport of peptides into the endoplasmic reticulum for binding to the groove of MHC class I molecules.

9.3 Predominantly antibody defects

The defect in several of the primary immunodeficiency diseases is restricted to antibody formation, either from impeded B-lymphocyte development or failure of effective B-lymphocyte responses to T-lymphocyte signals. This group of diseases, summarized in Table 2, presents clinically with recurrent pyogenic infections.

9.3.1 X-linked agammaglobulinaemia. This is the prototypic antibody deficiency. Affected males present in infancy or early childhood with recurrent pyogenic infections. The tonsils are small and lymph nodes are usually not palpable. Criteria for diagnosis include profound inability to make antibody and resultant low concentrations of all immunoglobulin isotypes. There is a decrease in circulating B lymphocytes (usually less than 5/1000 lymphocytes); plasma cells and germinal centres are absent. The number and function of T lymphocytes (including cell-mediated immunity) are unaffected. Pre-B cells are normally found in the bone marrow. The gene defect has been localized to the long arm of the X chromosome (Xq2l.3–22). XLA is due to mutations in a cytoplasmic tyrosine kinase designated *btk* or Bruton's agammaglobulinaemia tyrosine kinase. It consists of an N-terminal pleckstrin homology domain (PH), followed by a Tec (TH) domain, a Src homology 3, i.e. a SH3 domain, a SH2 domain and a C-terminal SH1 or tyrosine kinase domain. Mutations in all five domains of *btk* have been found in XLA. The xid mutation in mice is due to a missense mutation in which an arginine at residue 28 in the PH domain is converted to a cysteine. In female carriers of XLA the defective chromosome is preferentially lyonized during B-lymphocyte proliferation. The clinical phenotype may be very variable, even within the same family. Since the identification of the gene defect, it has been appreciated that the clinical phenotype is broader than originally conceived and all young males with a predominant antibody defect should be examined for mutations in *btk*.

9.3.2 Ig heavy chain deletion. Deletions and duplications in chromosome l4q32 of the heavy chain constant region genes occur in 5–10% of the Caucasian population and are probably common in all ethnic groups. Individuals who are homozygous for such deletions lack the relevant isotypes and subclasses. Heterozygotes often show slightly diminished levels of the affected subclasses. Most such families were found during the screening of entirely healthy, normal blood donors who had no history of recurrent infections. A few individuals homozygous for these defects have presented with recurrent pyogenic infections.

Deletions and point mutations affecting the expression of the surface-bound μ heavy chain have recently been found not only to cause a lack of IgM but also to lead to a defect in B-lymphocyte development resulting in agammaglobulinaemia. It is unlikely that this novel form of immunodeficiency can account for a proportion of female patients with a clinical picture resembling XLA.

9.3.3 κ chain deficiency. Two families have been described whose immunoglobulin chains have λ light chains only. No κ chains were found. Antibody formation was variable; circulating B lymphocytes were normal except that they did not carry κ light chain. Point mutations in the κ chain gene located at chromosome 2p11 were reported in one family.

9.3.4 Selective IgG subclass deficiency. Criteria for diagnosis should include normal total serum IgG levels with subnormal levels of one or more IgG subclasses. It is difficult to be certain of normal subclass levels. As noted in Section 6.2.1, the assays for subclasses are not well standardized; age-related and population-related norms are not always available; genetic variation exists among individuals in different ethnic groups. As IgGl is the predominant serum IgG subclass, deficiency of IgGl cannot generally occur without a decrease in total serum IgG, in which instance the defect should be considered as 'common variable immunodeficiency'. IgA levels are frequently, but not invariably, decreased in patients with IgG2 deficiency. Low levels of IgG3 are the most common IgG subclass abnormality reported in adults, whereas low levels of IgG2 are more common in children, particularly in association with poor responses to polysaccharide antigens. IgG4 levels vary widely in normal people, and many entirely normal people have no demonstrable IgG4 by standard techniques; selective deficiency of IgG4 alone is difficult to interpret. IgG2 deficiency, which is often associated with low or undetectable IgG4 levels and an inability to respond to polysaccharide antigens, may be confused with 'antibody deficiency with normal immunoglobulins'.

Selective immune globulin subclass deficiencies were shown to be present in healthy blood donors without undue susceptibility to infection. Deletions of immune globulin heavy-chain genes could be detected in these individuals.

9.3.5 IgA deficiency. About 1 in 700 Caucasians (in contrast to 1:18 500 Japanese individuals) have no demonstrable serum IgA. Many of these individuals have no apparent disease. Some people with recurrent sinopulmonary infections have been reported with entirely normal serum IgM and IgG levels, but absent or extremely reduced serum IgA levels. Whether the IgA deficiency or some other factors are involved in their illnesses is not clear. IgA deficiency is, however, more frequent in patients with chronic lung disease than in a normal age-matched population. Furthermore, IgA deficiency is more frequent in patients with autoimmune disease than in the normal healthy population. The defect is presumed to result from impaired switching or a maturational failure of IgA-producing lymphocytes. Autosomal recessive or dominant inheritance has been shown in some families. Fixed haplotypes of MHC genes are frequently associated with CVID and IgA deficiency and both disorders may reflect a continuum of the same underlying pathogenetic mechanism.

9.3.6 Selective antibody deficiency with normal immunoglobulins. It has been known for decades that some individuals selectively fail to respond to certain antigens. The characteristic defect is failure to respond to polysaccharide antigens. While most such people are normal, some have recurrent sinopulmonary infections. Criteria for diagnosis should include demonstrated failure to response to specific antigens, a normal response to other antigens and normal total serum IgG and IgM levels. In some of these people diminished serum IgG2 levels have been found. This appears to be an associative not causative relationship; IgG2 levels are not predictive of antibody responses. Antibody responses to polysaccharide antigens are often found to be diminished in people with sickle cell anaemia, asplenia (see Section 10), the Wiskott–Aldrich syndrome (Section 9.5.1) and the DiGeorge syndrome. In uncontrolled case studies, patients nonresponsive to polysaccharide antigens with normal immunoglobulins and chronic sinopulmonary disease benefited from IgG replacement. Nonresponders to polysaccharide antigens produce antibody well with conjugate vaccines. Some individuals who are not responsive to hepatitis vaccine and other protein antigens may fall into this category.

9.3.7 Common variable immunodeficiency (CVID). The term 'common variable immunodeficiency' (CVID) is used to describe an incompletely defined syndrome characterized by defective antibody formation. The diagnosis is otherwise based on exclusion of other known causes of humoral immune defects. The term 'acquired immunodeficiency syndrome' (AIDS) should be reserved for patients in whom the diagnosis of HIV infection has been established.

Perhaps because it has not yet been differentiated into its many probably distinct component syndromes, CVID is one of the most frequent of the primary specific immunodeficiency diseases: the incidence has been estimated at 1:10,000 to 1:50,000. Affecting males and females equally, the usual age of presentation is the second or third decade of life.

In common with all primary immunodeficiencies affecting humoral immunity, the clinical presentation of CVID is generally that of recurrent pyogenic sinopulmonary infections. Early diagnosis is important; some patients are only discovered when they have significant chronic lung disease, including bronchiectasis.

As with XLA, some patients develop unusual enteroviral infections with a chronic meningo-encephalitis, and other manifestations including a dermatomyositis-like syndrome. Patients with CVID are also highly prone to gastrointestinal infections caused by *Giardia lamblia* and *Campylobacter jejuni*.

There is an unusually high incidence of lymphoreticular and gastrointestinal malignancies in CVID. Lymphoproliferative disorders are often apparent from physical examination where, in contrast to XLA, up to a third of CVID patients have splenomegaly and/or diffuse lymphadenopathy. The lymph nodes show a striking reactive follicular hyperplasia. Noncaseating granulomas resembling sarcoidosis and striking nonmalignant lymphoproliferation occur. The gastrointestinal tract may also be involved in this process with a characteristic nodular lymphoid hyperplasia. Malabsorption with weight loss and diarrhoea and associated changes such as hypoalbuminaemia, vitamin deficiencies and other findings resembling celiac sprue are seen. Chronic inflammatory bowel diseases occur with increased frequency. Patients with CVID are prone to a variety of other autoimmune disorders (e.g. pernicious anaemia, haemolytic anaemia, thrombocytopenia and neutropenia).

The *sine qua non* for the diagnosis of CVID is defective antibody formation. These are usually accompanied by decreased serum IgG and IgA levels and generally but not invariably decreased serum IgM. Because CVID is a diagnosis of exclusion, those patients with elevated or high normal levels of serum IgM should be evaluated for the hyper-IgM syndrome (see Section 9.2.2). Male patients with very low or undemonstrable IgG, especially if they have markedly diminished numbers of circulating B cells, should be evaluated for XLA (see Section 9.3.1). In some patients CMI may be impaired with diminished T-cell function, and absent delayed-type hypersensitivity (DTH); the immunodeficiency under these circumstances involves both cellular and humoral immunity and the disease could be considered as a 'combined immunodeficiency' although the clinical expression is primarily defective antibody production.

As noted in Section 9.3.5, IgA deficiency is common in the general population. In CVID, IgA levels are undetectable or markedly below the normal range in almost all patients. Family members may also have an unusually high incidence of IgA deficiency. In addition, families of patients with CVID have an increased incidence of autoimmune disorders, auto-antibodies (including antilymphocyte antibodies) and malignancies, suggesting a wide expression of immune dysregulation. As would be expected in a heterogeneous group of undifferentiated diseases, various inheritance patterns for CVID (autosomal recessive, autosomal dominant, X-linked) have been noted. Sporadic cases with no obvious inheritance pattern are, however, the most common. In multiplex families containing several persons with CVID and IgA deficiency, involved individuals often inherit characteristic MHC alleles.

Many studies to identify the immunological defect(s) have been published. None to date has provided patterns sufficiently consistent for classification. There is no convincing evidence for any intrinsic B-cell defect of immunoglobulin genes, synthesis or secretion. While B cells (defined as CD19+) may be reduced in number, with appropriate stimulation they produce and secrete immunoglobulins.

CVID patients commonly have reduced CD4/CD8 ratios, with a reduction in CD4+ CD45RA+ ('unprimed') T cells and this suggests that there has been activation of T cells. The reported increased levels of IL-4 and IL-6, soluble CD8, CD25, β2-microglobulin, HLA-DR, LAF-3 and ICAM-1 are probably secondary to infection.

About 60% of CVID patients have diminished proliferative responses to T-cell receptor stimulation, and decreased induction

of gene expression for IL-2, IL-4, IL-5 and IFNγ. There is no evident abnormality of the T-cell receptors: T-cell receptor gene analyses indicate normal heterogeneity of gene rearrangements. There is decreased IL-2 production after T-cell receptor stimulation, which is correlated with diminished CD40 ligand expression. The abnormality appears to reside in CD4+ T cells and can be overcome by stimulating T cells with PMA and ionomycin – an alternative T-cell activation pathway (see Section 5). This is consistent with defective signal transduction, which could explain the diminished humoral immunity.

9.3.8 Non X-linked hyper-IgM syndrome. See Section 9.2.2.

9.3.9 Transient hypogammaglobulinaemia of infancy. Maternal IgG is actively transferred to the fetus throughout pregnancy. The serum IgG level of full-term infants is equal to or slightly greater than that of the mother. Maternal IgG in the infant disappears after birth with a half-life of 25–30 days and the infant's own Ig production is initiated, starting with IgM and followed by IgG and then IgA. The time of initiation and the rate of production of Ig by infants varies considerably. During the first 3–12 months of life in premature infants (where the transfer of maternal IgG is often limited) and in some full-term infants (particularly in families with immunodeficiency), the nadir of serum Ig concentration may be very low – within an 'immunodeficient' range. The initiation of antibody production may be delayed for as long as 36 months and ultimately is manifested by increased levels of serum IgG. Antibody production by the infants themselves can usually be documented by serial measurement of serum IgG levels and of antibody responses to vaccine antigens.

9.4 Predominantly T-cell defects

In addition to the ID diseases listed in Tables 1, 2 and 3, other primary defects in the immune system where the genetics and pathogenesis of the ID are not yet completely understood have been described in isolated cases and are listed in Table 8.

9.4.1 Primary CD4 T-cell deficiency. Profound, persistent decrease in circulating CD4+ T lymphocytes, with defective CMI, has been documented in patients, not infected by HIV, who present with opportunistic infections, such as cryptococcal meningitis and oral candidiasis. Immunoglobulin levels may be normal or slightly decreased. The pathogenesis and genetics of this abnormality are not yet known. When such patients are identified, CD4 enumeration should be carried out in other family members.

9.4.2 IL-2 deficiency. Children with SCID and normal circulating T-cell numbers were found to be unable to transcribe the IL-2 gene. The inheritance of the defect could not be determined.

9.4.3 Multiple cytokine defect. Children with SCID who were deficient in IL-2, IL-4, IL-5 and interferon-γ have been described. T cells lacked the nuclear factor of activated T cells (NFAT) promoter. The genetics of the defect are not yet known.

Table 8. Other primary immunodeficiency diseases

Primary CD4 deficiency
IL-2 deficiency
Multiple cytokine deficiency
Signal transduction deficiency ± myopathy
Calcium flux deficiency with myopathy

9.4.4 Signal transduction defect. A few children with SCID or CID fail to show normal calcium flux and diacylglycerol generation after antigenic stimulation of their T cells. The defect can be circumvented by stimulation with PMA or aluminium tetrafluoride (AlF_4). The genetics of this condition are not known and the precise defect(s) is not well characterized.

9.4.5 Calcium flux defect. Defective T-cell activation was found associated with an exquisite abnormality in calcium influx from the extracellular milieu in three patients with combined immunodeficiencies. Althugh the Ca^{++} flux defect is detected in all types of cells, phenotype is restricted to a T-cell deficiency. Molecular identification of the defect is still awaited.

9.5 Immunodeficiencies associated with other major defects

There are a variety of diseases in which immunodeficiency is an important but not exclusive component. Included in this Section (see Table 3) are those diseases where immunodeficiency is the dominant manifestation in syndromes with other defects.

9.5.1 Wiskott–Aldrich syndrome. This X-linked disease presents in infancy or early childhood. Clinical manifestations include eczema, recurrent, often unusual or unresponsive infections, and thrombocytopenia. The platelets are small. Surface sialoglycoproteins, CD43 and gpIb, and other sialoglycoproteins are unstable in the membranes of leucocytes and platelets. The lymphocytes have a characteristic 'bald' appearance on scanning electron microscopy. The cytoskeleton in the T cells and platelets is abnormal and the actin in these cells does not bundle normally. The proliferative response of the T cells to anti-CD3 is absent or greatly diminished. Serum immunoglobulins may at first be normal, but a progressive decrease of IgM develops. Antibody production, especially but not exclusively to polysaccharide antigens, is impaired. Progressive lymphopenia, most marked in the T-lymphocyte series with resulting defective CMI, develops. Autoimmune diseases including severe vasculitis and glomerulonephritis may be present. Death occurs in late childhood or in the second to fourth decades of life, often from lymphoma. The defective gene is on the short arm of the X chromosome at Xp11.22 and is selected against during differentiation of all blood cells; thus carrier detection is possible. The gene has been cloned and encodes a protein of 502 amino acids, which has been called the Wiskott–Aldrich syndrome protein (WASp). Its function is not well understood but it appears to be involved in signal transduction for cytoskeletal reorganization. Mutations in the WASp gene also cause so-called X-linked thrombocytopenia.

9.5.2 Ataxia-telangiectasia. This autosomal recessive syndrome is characterized by progressive cerebellar ataxia, the appearance of fine telangiectases, especially on ear lobes and conjunctival sclera, and, eventually, in most patients, recurrent sinopulmonary infections. Raised levels of serum alpha fetoprotein are present in 95% of patients. Immunodeficiency, while not invariably demonstrable in the early life of affected people, develops in at least 70% of cases. There is no consistent immunological pattern; no single abnormality has been found to exist in all patients. Serum Ig is decreased in varying patterns: IgG2, IgG4, IgA and IgE are commonly low or absent. Antibody responses to polysaccharide and protein antigens may be reduced. The numbers and function of circulating T lymphocytes, including DCH, are generally diminished. There is an increased incidence of autoantibodies.

Cells from patients with ataxia-telangiectasia have a disorder of their cell cycle checkpoint pathway that results in an extreme hypersensitivity to ionizing radiation. Lymphocytes show frequent

chromosomal breaks, inversions and translocations involving sites of the T-cell receptor genes and immunoglobulin gene complexes on chromosomes 7 and 14. In fibroblasts chromosomal breaks, inversions and translocations are random. A-T patients and their parents have a strikingly increased susceptibility to malignancies. Breast cancer in female A-T carriers is reported to be increased. The overall risk of cancer in heterozygotes generally is probably also increased. Death in patients usually occurs in early adult life after years of increasing disability from pulmonary disease or (often lymphoreticular) malignancy. A-T cells fail to upregulate p53 expression following DNA damage by irradiation, indicating that the A-T protein functions upstream from p53 and plays a major role in sensing, but not in repairing, double-stranded DNA breaks.

The gene *ATM* (for A-T *mutated*) was isolated in 1995 by positional cloning. More than 150 mutations have been identified; 80% of these result in truncation of the protein. Most of these mutations are unique. Most patients are compound heterozygotes. The gene product has strong homology to phosphoinositide 3-kinase and preliminary results confirm a protein kinase activity. *ATM* knockout mice show defects in T-cell maturation and develop lymphoid tumours similar to those seen in patients, especially malignant thymic lymphomas. The mice die at 2–4 months with some neurological dysfunction but few if any signs of ataxia.

The Nijmegen Breakage Syndrome (NBS) overlaps with that of A-T; patients are radiosensitive, immunodeficient, cancerprone and similarly manifest reciprocal translocations involving chromosomes 7 and 14. However, they do not have ataxia, telangiectasia nor telangiectases. They are mentally retarded and microcephalic. Family studies do not link the NBS gene to chromosome 11q23.1, the site of the *ATM*. The $A–T_{Fresno}$ syndrome overlaps both classical A-T and NBS, including immunodeficiency and an elevated AFP.

For several syndromes with immunodeficiency and chromosomal instability see Section 10.1.

9.5.3 The DiGeorge anomaly. The DiGeorge anomaly is one of a series of contiguous gene syndromes that affect multiple organs during early embryogenesis. Almost all (80–90%) patients with the DiGeorge anomaly have deletions (often microdeletions) of 22q11-ter. There are several other syndromes with deletions located to the same area. Because they all involve deletions of 22q11-ter they have been termed 'CATCH 22', an acronym for the involved organs: cardiac abnormalities, abnormal facies, thymic hypoplasia, cleft palate and hypocalcaemia. This group of syndromes would include the velocardiofacial (Shprintzen) syndrome, the conotruncal anomaly face syndrome, Cayler syndrome and some patients with Opitz GBBB syndrome. Additional cases of the DiGeorge anomaly may derive from 10p deletions, from the fetal alcohol syndrome, retinoic embryopathy or maternal diabetes. The characteristic pathological manifestations include multiple anomalies of the third and fourth branchial arch derivatives; Type 1 truncus arteriosus, dysmorphic facies with micrognathia, thymic and parathyroid hypoplasia or aplasia. Clinically, neonatal tetany and/or cardiac failure are the presenting manifestations in most affected infants. The facial features then arouse suspicion as to the diagnosis.

Infections are usually not a presenting manifestation. Even though the thymus is frequently involved, only about 20% of those with the anomaly have decreased numbers and function of T lymphocytes. At autopsy the thymus is small and atrophic, often containing ectopic lobes that are normal in appearance. Surviving infants over time may naturally acquire functional T cells and the immunodeficiency becomes corrected. It is therefore difficult to assess the value of the various treatment regimens that have been attempted.

9.5.4 Chediak–Higashi syndrome (CHS). This autosomal recessive disease is characterized by partial oculo-cutaneous albinism due to dysmaturation of melanosomes. Giant granules are found in all nucleated cells. Neutropenia, abnormalities of granulocyte and monocyte mobility and chemotaxis, as well as defective NK-cell cytotoxicity, are demonstrable. The latter may explain an EBV-associated lymphoproliferation resembling familial lymphohistiocytosis (FLH) that leads to pancytopenia and hypofibrinogenemia following macrophage activation. The CHS gene has been identified and encodes a protein which may regulate microtubule-mediated lysosome transport.

9.5.5 Partial albinism and immunodeficiency (Griscelli Syndrome). This rare autosomal recessive disease is characterized by partial albinism due to abnormal migration of melanosomes from melanocytes to keratinocytes. It is distinguished from Chediak–Higashi syndrome by the absence of giant granules. Patients have a propensity for fungal, viral and bacterial infections. Immunoglobulins and DTH may be decreased. Abnormal T-cell cytotoxicity and diminished NK-cell activity have been described. These patients have, in addition to increased susceptibility to infection, a lymphoproliferative reaction similar to that seen in Chediak–Higashi syndrome that leads to early death. The defect has been corrected by bone marrow transplantation.

9.6 Associated conditions

In addition to the infections of the respiratory and gastrointestinal tracts noted previously, patients with primary specific immunodeficiency are particularly prone to several other conditions.

9.6.1 Malignancies. Age-specific mortality rates for cancer in patients with primary ID exceed by 10–200 times the expected rates for the general population. The majority of cancers are observed in patients with ataxia-telangiectasia and the Wiskott–Aldrich syndrome. The causal relationship is exemplified by the finding that treatment of patients with SCID or Wiskott–Aldrich syndrome by bone marrow transplantation from an MHC-matched sibling donor has led to an impressive reduction in this susceptibility to malignancy.

In patients with ataxia-telangiectasia, transpositions, inversions and breaks of chromosomes 7 and l4 at the sites of the T-cell receptor are associated with lymphoreticular malignancies. These patients also develop malignancies of rapidly replicating cells in other organs.

The types of tumour, lymphoreticular malignancies, in all groups of ID are different from those observed in nonselected populations. Some have clear evidence of clonal proliferation, some are associated with Epstein–Barr virus infection. It appears likely that there is an association between ID and oncogenesis. Possible mechanisms include defective immunological surveillance; defective immune response to oncogenic viruses; chronic overstimulation or proliferation of responsive cells to antigens; independent effects of the same common cause (i.e. chromosomal instability in ataxia-telangiectasia). In CVID there is an increase in the incidence of lymphomas and in gastrointestinal malignancies.

Papilloma virus infections also appear to be more frequent in patients with ID with the production of local verrucae, condylomata acuminata and localized, usually genital, intraepithelial neoplasia.

9.6.2 Autoimmunity. Several autoimmune syndromes have been described in association with ID. These include pernicious anaemia, autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpura, systemic lupus erythaematosus, thyroiditis, Sjögren's syndrome, chronic active hepatitis and myasthenia gravis. In addition to autoantibodies against blood cells, autoantibodies to immunoglobulins and various tissue antigens have been observed. Inflammatory bowel disease is a frequent complication of ID; whether the cause is infectious or autoimmune is not always clear.

9.6.3 Atopic allergy. Atopic allergy due to the presence of IgE antibodies to inhaled, ingested or injected proteins affects 15–20% of the general population. It gives rise to clinical symptoms of asthma, rhinitis, eczema, urticaria and anaphylaxis. Patients with primary immunodeficiencies often have symptoms consistent with asthma and/or rhinitis. In many of the cases this is due to chronic non-IgE-mediated inflammation. However, attempts should be made, particularly in patients who retain the capacity to make a partial antibody response, to rule out an allergic aetiology or contribution. This is achieved by performing immediate hypersensitivity skin tests and by measuring IgE antibodies in the serum.

9.6.4 Unusual viral infections. Patients with predominantly antibody defects (particularly with X-linked agammaglobulinaemia) are especially susceptible to chronic viral infections with ECHO or other enteroviruses. This is characteristically a meningoencephalitis or a dermatomyositis-like syndrome. Such patients may shed virus for years. Virus may be isolated from cerebrospinal fluid and at postmortem from all viscera. Left untreated, the infection is fatal. High doses and specific intravenous IgG have controlled these infections in some patients.

Several patients with primary ID have been infected with HIV and with hepatitis C virus. Testing for seroconversion to HIV or hepatitis C virus is of little or no use in diagnosis because of the intrinsic inability of patients with ID to make antibody. PCR techniques should be used to ascertain the presence of viral genomic material. It has also been reported that some CVID patients infected with HIV or hepatitis C have shown some recovery of immunoglobulin levels.

9.7 Treatment of specific immunodeficiency

9.7.1 Bone marrow transplantation. Transplantation of bone marrow cells from HLA genotypically identical donors (i.e. matched sibling donors or other HLA identical members of a family) has led to complete immunological reconstitution of most patients with SCID, including those with ADA deficiency, and those with reticular dysgenesis. Bone marrow transplantation has also been successful in the Wiskott–Aldrich syndrome, leucocyte adhesion deficiency, MHC class II immunodeficiencies, Kostmann's syndrome, chronic granulomatous disease, X-linked hyper-IgM and Chediak–Higashi syndrome. Ideally, donor and recipient should be identical at the HLA-A, B, C and DR loci. Unfortunately, 75% of patients do not have a compatible donor. Great progress has been made in haplo-identical bone marrow transplantation in recent years. Extensive conditioning of the recipient to prevent rejection and the elimination of T cells from the donor cells to avoid GvH disease are mandatory. Success with T-cell engraftment has been very encouraging, but it has been difficult to establish B-cell engraftment with haplo-identical bone marrow. Full reconstitution takes longer than engraftment with HLA identical donors.

An increasing number of successful bone marrow transplants are being performed using bone marrow or peripheral blood stem cells from unrelated donors identified in one of the registries of HLA typing. Attention is being paid to HLA-D match. Stem cells from umbilical cord blood stored in certain centres are being used in an increasing number of cases.

Peripheral blood stem cells from specially pretreated donors after T-cell depletion of the donated cells with or without further purification of the stem cell population is increasingly used in cases of unrelated histocompatible and haplo-identical situations. Intrauterine transplant of parental haematopoietic stem cells has led to successful T-cell engraftment in some cases of X-linked SCID.

Acute GvH) disease, when it occurs, generally appears 10–14 days after a transplant and is usually manifested by fever, Coombs' test-positive haemolytic anaemia, erythematous, maculopapular skin rash, bloody diarrhoea, hepatosplenomegaly, aregenerative pancytopenia and death. The various means proposed to prevent GvH disease have included the use of cyclosporin A, alone or together with methotrexate. T-cell depletion of donated bone marrow has also been used. Persistent low-grade GvH reactions, characterized by hepatomegaly, jaundice or skin rashes, can continue for many months and become chronic and severely debilitating.

The establishment of immune competence ('take' of the graft) may be identified by: improvement of clinical status (e.g. weight gain, rapid resolution of moniliasis); appearance of T and B cells in the circulating blood; demonstration of donor cells in the recipient by genetic markers, including enzyme activity in previously deficient patients; increase of immunoglobulin levels (including Ig of donor origin); appearance of humoral antibodies (including those following antigenic stimulation); return of C1q level to normal; and appearance of CMI reactions. Of these, the establishment of chimerism is the most reliable evidence of engraftment. Appropriate tests for mosaicism include sex and other chromosomal studies, molecular analysis at polymorphic loci, HLA and red cell antigens, plasma protein or enzyme allotypes.

Tests of immunological competence should be repeated periodically in successful cases, because subsequent gradual decline of function has been observed in some instances of initially successful engraftment. Children dramatically restored immunologically have also occasionally died of pre-existing pulmonary infections with *Pneumocystis carinii* or other organisms just after immunological capacity has been restored. Prophylactic treatment with sulphamethoxazole-trimethaprim has proven useful in the treatment of these complications. Several deaths from varicella have occurred in successfully transplanted ID patients; such patients should be passively protected with varicella/zoster immune globulin (VZIG) and acyclovir following exposure, if no circulating antibody can be demonstrated. CMV has been successfully treated with gancyclovir and foscarnet and a high dose of specific immunoglobulin. Other antiviral agents are being developed and tested at the present time. Many SCID patients who do not have B-cell engraftment (~40%) require IgG replacement.

The risk of developing EBV-induced B-cell lymphomas in transplant recipients, particularly of haplo-identical bone marrow donations, has been a difficult and as yet unsolved problem.

9.7.2 Replacement of immunoglobulins. The efficacy of immunoglobulin replacement for antibody deficiency syndromes was well established in the 1950s. It is now accepted that all patients with primary specific immunodeficiency who have significantly diminished serum IgG levels and/or demonstrated defects in antibody production should receive IgG replacement. Preparations suitable for either intramuscular or intravenous use are available for this purpose; the intramuscular preparations should never be

given intravenously, but they can be given subcutaneously. Intravenous immunoglobulin replacement is the preferred treatment. Standards for the preparations are the subject of an IUIS/WHO report (Bull. WHO 60 (l), 43, 1982). Viral partition and inactivation methods used during the fractionation procedures have been incorporated into the production of immunoglobulin and all manufacturers are required to provide data that validate the viral inactivation methods used. Thus, HIV and other retroviruses are effectively excluded by current fractionation procedures, essentially eliminating the risk for transmission of lipid-coated viruses. Clusters of hepatitis C infections have been reported in patients who received certain batches of IVIG.

Experience has shown that replacement therapy with intravenous Ig is life saving. If replacement is started early, and if appropriate amounts are given with sufficient frequency, the cycle of recurrent infections and progressive lung damage can be arrested. Near-normal serum IgG levels can be maintained with ease; general experience suggests that 400–500 mg/kg/month may be sufficient to prevent breakthrough infections. It has been documented that abnormal pulmonary function may improve, even if chronic lung damage is present, if doses of IgG > 600 mg/kg/month are given.

Preparations of IgG for replacement contain predominantly IgGl and IgG2; the amounts of IgG4 in most preparations are small, and in some IgG3 is absent. Nevertheless patients with selective IgG subclass deficiency, with or without IgA deficiency, may benefit from IgG replacement which provides high titres of antibodies that such a patient is unable to make. Neither the precise indications nor the dosage for such therapy have been well established.

Immunoglobulin replacement therapy by subcutaneous infusions of gammaglobulin is used increasingly. The results indicate that this type of treatment is well tolerated with a very low frequency of systemic adverse reactions.

Untoward reactions to infusions of immunoglobulin may occur. These include dyspnoea, flank pain, hypotension, collapse, fever, rashes or rigours. Severe wheeze, chest pain or symptoms of anaphylaxis are indications for the infusion to be stopped and treatment to be given immediately (antihistamines and hydrocortisone i.v. or adrenaline s.c.). Most reactions are probably due to immunoglobulin aggregates, with only a very few being attributable to antibodies to IgA. Reactions tend to occur more frequently in severely hypogammaglobulinaemic patients, particularly at the initiation of treatment, and in those with intercurrent infections. Many reactions can be traced to excessively fast rates of infusion.

9.7.3 Enzyme replacement. Partial replacement of enzymes with frozen irradiated red blood cells has been attempted in infants with ADA or PNP deficiency. Apparently, the amounts of purine degradation enzymes within the red cells are not sufficient to permit efficient degradation of toxic metabolites within lymphocytes. Partial enzyme replacement in ADA deficiency has also been attempted by the use of bovine ADA modified by conjugation with polyethylene glycol. Repeated weekly administration of the conjugated enzyme resulted in marked clinical and immunological improvement in several patients.

Since the gene for ADA has been cloned, it has become possible to express it in T cells with a retroviral vector. This has provided the basis for an attempt at gene therapy in patients with ADA deficiency. This therapeutic approach is at present still under study. Transplantation of HLA-identical bone marrow remains the treatment of choice.

9.7.4 Blood transfusions. Blood transfusions should never be given to patients with cell-mediated immunodeficiency, unless fully oxygen-saturated blood has been irradiated to eliminate viable white blood cells that may inappropriately engraft the patient. Blood transfusion is also safe when processed by freezing and centrifugation. However, lymphocytes are still viable in outdated blood, washed red blood cells, unprocessed plasma, and platelet preparations.

9.7.5 Treatment of opportunistic infections. Individual infections should be treated early with full doses of antimicrobial agents. Where possible, narrow-spectrum drugs selected on the basis of microbial sensitivity testing should be used. While prophylactic antibiotics may be useful, they increase the hazard of infection with fungi or other resistant organisms. Long-term treatment with combination sulfa drugs (cotrimoxazole, sulphamethoxazole-trimethoprim) and itrakonazole is believed to be of some benefit, but this has not been critically evaluated.

Antiviral agents such as acyclovir and gancyclovir have proven valuable in the treatment of some patients with persistent or severe viral infections.

9.7.6 Gastrointestinal disorders. Intestinal disease is frequent in ID patients and, in addition to treatment of infection or infestation, disaccharide or gluten-free diets may be of benefit in patients with sprue-like symptoms. In some instances intravenous hyperalimentation may be justified. *Giardia lamblia* and *Campylobacter* are frequent causes of diarrhoea, steatorrhoea or weight loss in ID. Treatment with atabrine or metronidazole is effective for giardiasis.

9.8 Immunodeficiency associated with lymphoproliferative disorder (see Table 9)

This syndrome (also called the Canale–Smith syndrome) is characterized by diffuse lymphadenopathy with hepatosplenomegaly, hypergammaglobulinemia, autoimmune cytopenias, and immune complex glomerulonephritis. Flow cytometry of peripheral blood lymphocytes reveals markedly increased B cells (5–20-fold), NK cells, and the expansion of a population of $TCR\alpha\beta^{+}CD4^{-}CD8^{-}$ cells which have impaired proliferative and cytokine responses to TCR engagement. This population constitutes between 15 and 70% of peripheral blood T cells in these patients.

Autoimmune lymphoproliferative syndrome (ALPS) results from mutations in the gene encoding the Fas (CD95) molecule, a member of the tumour necrosis factor receptor family of membrane molecules, and an important regulator of apoptosis following T-cell activation. The disease is expressed in heterozygotes, indicating the importance of other host factors in disease expression. This disease is similar to syndromes arising as spontaneous mutations in the *lpr* and *gld* mouse strains. The *lpr* defect corresponds to the murine CD95 homologue, while *gld* encodes the CD95 ligand.

Ligation of CD95 initiates signals transduced along a pathway

Table 9. Immunodeficiency associated with lymphoproliferative disorder

1. Fas deficiency
2. Fas ligand deficiency
3. FLICE or Caspase 8 deficiency
4. Unknown

including the molecules FLICE and caspase 8. Targeted disruption of these genes in mice results in embryonic lethal phenotypes.

10 IMMUNODEFICIENCY ASSOCIATED WITH OR SECONDARY TO OTHER DISEASES

Table 10 lists some of the many congenital and hereditary conditions in which immunodeficiency has been described.

10.1 Chromosomal instability and defective repair

The immune system is dependent upon rapid and accurate lymphocyte differentiation and replication. Any syndrome associated with chromosomal instability or defective repair, such as ataxia-telangiectasia (Section 9.5.2) can be expected to have associated immunological defects. The following syndromes fall into this category.

10.1.1 Bloom syndrome. Low birth weight, retarded growth, rashes from light sensitivity, well demarcated hyper- and hypopigmented skin lesions, molar hypoplasia and facial telangiectasia, characterize this rare autosomal recessive chromosomal instability syndrome that has been mapped to 11q23. Immunodeficiency with frequent infections, increased susceptibility to malignancies, reduced T-cell function and decreased serum IgM are found. Occasionally serum IgG and IgA may also be diminished. IgM + B cells are normal in number; the defect appears to be in B-cell maturation to IgM secretion. NK cell defects have been described.

10.1.2 DNA ligase 1 defect. A patient with growth retardation, sun sensitivity, immunodeficiency and defective DNA repair, phenotypically similar to Bloom syndrome (Section 10.1.1) was found to have mutations in the DNA ligase 1 gene. The defect was not found in cells from patients with Bloom syndrome. The DNA ligase 1 defect thus appears to be a distinct entity.

10.1.3 Xeroderma pigmentosum. This is another photosensitive dermatosis. From infancy patients with this rare autosomal recessive condition have a marked sensitivity to sunlight and develop striking skin lesions – erythema, bullae, telangiectasia, keratoses, basal and squamous cell carcinomas. They are unable to repair UV damage to their DNA. A small number (> 5%) of the affected children have recurrent infections and a demonstrable immunodeficiency with a decrease in CD4+ cells. The sera reportedly contain antibodies which suppress T-cell (and possibly NK-cell) function. Serum IgG levels may be diminished. Cockayne's Syndrome and Trichothiodystrophy, similar photosensitive dermatoses, have not yet been described as having any immune defect.

10.1.4 Fanconi anaemia. This autosomal recessive syndrome is characterized by multiple organ defects including bone marrow failure, hyperpigmentation and cafe au lait spots, limb defects (radial hypoplasia), genitourinary anomalies, abnormal facies

Table 10. ID associated with or secondary to other congenital or hereditary conditions

Chromosomal instability or defective repair
Bloom syndrome
Xeroderma pigmentosum
Fanconi anaemia
ICF syndrome
Seckel ('bird-headed' dwarfism) syndrome

Chromosomal defects
Down syndrome (Trisomy 21)
Turner syndrome
Deletions or rings of chromosome 18 (18p– and 18q–)

Skeletal abnormalities
Short-limbed skeletal dysplasia (short-limbed dwarfism)
Cartilage-hair hypoplasia (metaphyseal chondrodysplasia)

Immunodeficiency with generalized growth retardation
Schimke immuno-osseous dysplasia
Dubowitz syndrome
Kyphomelic dysplasia with SCID
Mulibrey's nannism
Growth retardation, facial anomalies and immunodeficiency
Progeria (Hutchinson–Gilford syndrome)

Immunodeficiency with dermatological defects
Ectrodactyly ectodermal dysplasia–clefting syndrome
Immunodeficiency with absent thumbs, anosmia and ichthyosis
Dyskeratosis congenita
Netherton syndrome
Anhidrotic ectodermal dysplasia
Papillon–Lefèvre syndrome
Congenital ichthyosis

Hereditary metabolic defects
Acrodermatitis enteropathica
Transcobalamin 2 deficiency
Type 1 hereditary orotic aciduria
Intractable diarrhoea, abnormal facies, trichorrhexis and immunodeficiency (?Stankler syndrome)
Methylmalonic acidaemia
Biotin-dependent carboxylase deficiency
Mannosidosis
Glycogen storage disease, Type 1b

Hypercatabolism of immunoglobulin
Familial hypercatabolism
Intestinal lymphangiectasia

Other
Hyper-IgE syndrome (Job syndrome)
Chronic muco-cutaneous candidiasis
Hereditary or congenital hyposplenia or asplenia
Ivermark syndrome
Familial intestinal polyatresia

(microphthalmia, micrognathia, broad nasal base, epicanthal folds) and chromosomal breaks. There is an increased incidence of leukaemia. Decreased T-lymphocyte and NK-cell function and decreased serum IgA concentrations have been described.

10.1.5 ICF syndrome. ICF is an acronym for the immunodeficiency, centromeric instability, facial anomalies that characterize this syndrome. It is readily recognized by characteristic ocular hypertelorism, flattened nasal bridge, epicanthic folds, tongue protrusion and micrognathia. The immunodeficiency presents with recurrent sinopulmonary, gastrointestinal and skin infections. Generally, but not uniformly, serum IgM, IgG and IgA are decreased. Combined immunodeficiency has been described. Immunoglobulin administration may reduce infections in some patients. Variable degrees of mental retardation occur. The diagnostic finding is abnormal condensation of heterochromatin in chromosomes 1, 9, and 16 with increased frequency of mitotic recombination and the formation of multibranched chromosomes. This is associated with localized hypomethylation of classical satellite DNA. Inheritance is probably AR.

10.1.6 Seckel ('bird-headed' dwarfism) syndrome. This is another one of the many 'bird-like' facies syndromes reported to be associated with immunodeficiency. Seckel syndrome is characterized by dwarfism (intrauterine in onset), a 'bird-head' facies, severe brain dysplasia, mental retardation and many skeletal anomalies. Increased chromosomal breakage has been described. Some of the affected individuals develop hypoplastic anaemia, pancytopenia and decreased serum immunoglobulins.

10.2 Chromosomal defects

Of the many syndromes associated with stable chromosomal abnormalities, several are accompanied by immunodeficiency.

10.2.1 Down syndrome. Trisomy 21 (Down syndrome) is a relatively common condition characterized classically by dysmorphic facies with slanting palpebral fissures, a flattened occiput, flat nasal bridge, hypotonia, mental retardation (which may be very mild) and recurrent infections. There is a progressive decrease in serum IgM. The thymus may be dysplastic. An increase in CD8 T cells with an NK phenotype has been described; the NK cell activity, however, was low. Abnormal delayed hypersensitivity (CMI), antibody formation and cytokine production have been reported. Chromosome 21 carries the gene encoding the interferon receptor; trisomy 21 lymphocytes are more sensitive to interferon than normals. As patients with Down syndrome often have evidence of early ageing and because the immune dysfunction may be progressive, it has been postulated that the immune defect may represent 'early immunological senescence'. The phenotypic findings of Down syndrome are perhaps best explained by a contiguous gene defect.

10.2.2 Turner syndrome. These patients, who have generally an XO karyotype, present clinically with short stature, ovarian dysgenesis, transient lymphoedema, a webbed neck and broad chest. They often have recurrent infections, autoimmune diseases and increased numbers of malignancies. About 50% have immunodeficiency, with decreased serum IgG and IgM. T- and B-cell numbers and responses are usually within normal limits. Patients with variants of the Turner syndrome, including mosaics, may show the same features.

10.2.3 Deletions or rings of chromosome 18 (18p− and 18q−). Individuals with rings and/or deletions of the short or long arms of chromosome 18 may present with midfacial hypoplasia or ptosis, mental retardation, and/or growth deficiency. About 50% have been found to have markedly decreased serum IgA and some have IgG subclass deficiency and defective antibody formation.

10.3 Skeletal abnormalities

The known interrelationship between new bone formation, lymphocytes and cytokines leads to the expectation of some forms of skeletal dysplasia in patients with immunodeficiency. Short-limbed skeletal dysplasia (dwarfism) has, for example, been described in patients with ADA deficiency (see Section 9.2.1.2). In the syndromes listed below skeletal abnormalities are striking features; immunodeficiency is frequently although not universally present. All are also associated with growth-retardation such as that described in Section 10.4.

10.3.1 Short-limbed skeletal dysplasia (short-limbed dwarfism). The preferred nomenclature is short-limbed skeletal dysplasia (SLSD). The term is used to describe a group of patients in whom stature is disproportionately reduced, with greater involvement of the limbs than the trunk. It has been reported in patients with ADA deficiency (see Section 9.2.1.2) and in SCID with normal ADA (see Section 9.2).

10.3.2 Cartilage-hair hypoplasia (metaphyseal chondrodysplasia). These patients present with short-limbed skeletal dysplasia and usually, although not always, fine, sparse (hypoplastic) unpigmented hair and severe immunodeficiency. The inheritance is AR. In Finland, the incidence is approximately 1:23 000 births. Multiple organ systems may be involved: ligamentous laxity, macrocytic anaemia, neutropenia, megacolon (including Hirschsprung's syndrome) have all been described. Most patients have frequent infections and demonstrably defective cellular immunity. The defects in cellular immunity may relate to abnormal intracellular signalling pathways or a transacting factor which regulates the expression of several early activation genes in T cells. B-cell numbers and functions are normal. The gene has been mapped to 9p13. Bone marrow transplantation has fully corrected the immunodeficiency, but does not influence the chondrodystrophy.

10.4 Immunodeficiency with generalized growth retardation

Generalized growth retardation is common in children with recurrent infections, malnutrition and chronic pulmonary disease. It is prominent in syndromes involving the endocrine or the gastrointestinal tract. As noted in Sections 10.1 and 10.2, dwarfism is a common component of syndromes involving chromosomal abnormalities and those with skeletal dysplasia (Section 10.3). The following syndromes are characterized by growth retardation as a presenting condition.

10.4.1 Schimke immuno-osseous dysplasia. Several patients have been described with skeletal dysplasia, pigment abnormalities (lentigenes) and nephropathy. The inheritance is AR. Most patients have recurrent infections with striking lymphopenia, especially of T (CD4+) cells. Mitogen responses and DTH are diminished. B-cell numbers and function are normal. The nephropathy is associated with circulating immune complexes.

10.4.2 Dubowitz syndrome. This rare AR condition is associated with pre- and postnatal dwarfism, distinctive facial dysmorphism and eczema. Bone marrow failure with pancytopenia has been reported.

10.4.3 Hoyeraal–Hreidarsson syndrome. Two brothers and three additional unrelated boys with prenatal growth retardation, cerebellar hypoplasia, microcephaly, developmental delay and progressive pancytopenia with combined immunodeficiency have been reported.

10.4.4 Kyphomelic dysplasia with severe combined immunodeficiency. This rare skeletal dysplasia with short angulated femora, bowed long bones, short ribs and metaphyseal abnormalities has been described in an infant with SCID.

10.4.5 Ischiadic hypoplasia, renal dysfunction and immunodeficiency. A child of consanguineous parents with prenatal growth retardation, microcephaly, abnormal facies (flat-face, hypertelorism, epicanthic folds, strabismus, short nose, low set ears), syn- and polydactyly, ischiadic hypoplasia with hypospadias and cryptorchidism, renal dysfunction and hypogammaglobulinemia, which appears distinct from Dubowitz syndrome (Section 10.4.2), has been described.

10.4.6 Mulibrey's nannism. Mulibrey is an acronym for muscle, liver, brain and eye, organs described to characteristically be involved in this AR syndrome. Affected patients have pre- and postnatal dwarfism, a triangular facies (with a J-shaped sella turcica) and characteristic retinal pigment changes. Many have associated dermal lesions (naevi flammei, angiomata). Early death, commonly from constrictive pericarditis (which may be relieved by surgical intervention) occurs. Growth hormone and antibody deficiency has been described in this form of dwarfism. Serum IgM and IgG were decreased, antibody responses were ablated, and B-cell numbers decreased. Growth hormone administration resulted in increased growth but did not improve immunological responses.

10.4.7 Growth retardation, facial anomalies and immunodeficiency. A variety of other case reports suggest that the combination of facial anomalies and growth retardation may be associated with recurrent infection. In some instances there are decreased immunoglobulins, in some neutropenia. The reports are insufficient at this time to categorize the clusters more clearly, but the finding of facial anomalies with growth retardation warrants immunological investigation.

10.4.8 Progeria (Hutchinson–Gilford syndrome). Alopecia, short stature and loss of subcutaneous fat are the hallmarks of this rare syndrome. Skin fibroblasts have reduced ability to replicate. A described reduction in T (CD4+) cells and reduced IgG levels may relate to the rapidly accelerated ageing process. A somewhat similar and very rare condition, the Smith–Mulvihill syndrome (also known as Shepard–Elliot–Smith–Mulvihill) presents with short stature, progeria, microcephaly with ocular and dental anomalies, and pigmented naevi. In some instances there are recurrent infections and diminished IgG levels and in one patient lymphopenia with diminished T and B cells was found.

10.5 Immunodeficiency with ectodermal dysplasia and other dermatological defects

As noted in previous sections, immunodeficiency is often associated with a variety of dermatological conditions, including some described as ectodermal dysplasia, a term that encompasses many conditions that are often not clearly differentiated. The following syndromes present primarily as dermatological problems.

10.5.1 Ectrodactyly ectodermal dysplasia-clefting syndrome (EEC). These unusual and rare patients are recognized most often because of their clefting problems, lobster claw deformities of the extremities and cleft palate, that are present in the majority, but not all of the affected patients. The uniform finding is of ectodermal dysplasia involving the hair, skin, nails and teeth. Lacrimal duct atresia is common. They have recurrent respiratory, lachrymal and urinary tract infections. In one case a T-cell abnormality, which later resolved, was reported. A thymic abnormality resultant from the ectodermal defect was postulated. In other cases the immune system has been found to be normal and the recurrent infections thought to be secondary to the ectodermal defects per se. The ectrodactyly locus is at 7q21.3.

10.5.2 Immunodeficiency with absent thumbs, anosmia and ichthyosis. Several syndromes, for example the Fanconi syndrome (Section 10.1.4), are characterized by radial dysplasia and/or absent thumbs. Three sibships have been reported with short stature, absent thumbs, anosmia, ichthyosis (with chronic mucocutaneous candidiasis) and recurrent, predominantly viral and fungal as well as bacterial infections. Serum IgA was absent; IgG and IgM were variably decreased. Mitogen responses were diminished.

10.5.3 Dyskeratosis congenita. This disease is characterized by cutaneous pigmentation, nail dystrophy and oral leukoplakia. Inheritance can be X-linked, AR or AD. There is an increased risk of malignancy. Bone marrow failure frequently occurs in childhood with resultant increased infections, but variable immunological defects. Hypogammaglobulinaemia is found in many patients, along with diminished cell-mediated immunity.

10.5.4 Netherton syndrome. A large group of patients presenting with a classic triad of trichorrhexis (invaginata and nodosa), congenital icthyosis (linear circumflex, erythrodermia) and atopy have been described. The ichthyosis, which is present at birth, can be associated with profound hypernatraemic dehydration. Decreased *in vitro* lymphocyte responses to mitogens and negative skin tests to a battery of bacterial antigens may be found. Some have had abnormally low or high serum immunoglobulin levels.

10.5.5 Anhidrotic ectodermal dysplasia. This syndrome is characterized by hypohidrosis, faulty dentition and hypotrichosis. Most cases are X-linked recessive; a few are AR. Heterozygotic females may have partial symptomatology. Recurrent upper respiratory infection is a frequent problem. Although immunoglobulin levels and DTH have been described as abnormal in some patients, no consistent T- or B-cell abnormality has been found. Diminished chemotactic activity has been reported in a possibly related condition, congenital icthyosis (see also Section 10.5.7).

10.5.6 Papillon–Lefèvre syndrome. Hyperkeratosis of the hands and feet with periodontal disease leading to premature loss of teeth is, in some cases, associated with pyoderma. Neutrophil chemotaxis is often diminished. This syndrome needs to be distinguished from the leucocyte adhesion defects (Section 12.2.1) and the Hyper-IgE (Job's) syndrome (Section 10.8.1).

10.5.7 Congenital ichthyosis. The X-linked form of this disease is caused by a deletion in the steroid sulphatase gene on the short arm of the X chromosome, close to the gene associated with chronic granulomatous disease (see Section 12.3.1) at Xp21. Combined deletions of both occur.

10.6 Hereditary metabolic defects

Several hereditary metabolic defects other than adenosine deaminase and purine nucleoside phosphorylase deficiency can impair immune function. In the instances listed below the impairment of immune function may be only a minor component of the manifestations of the disease.

10.6.1 Acrodermatitis enteropathica. This autosomal recessive disease characterized by eczema, diarrhoea, and malabsorption has been reported in association with recurrent sinopulmonary infections, decreased serum Ig, intermittently reduced numbers and function of T cells and abnormal cell-mediated immunity. In some patients abnormal chemotaxis was found. The syndrome is attributable to zinc deficiency from defective gastrointestinal

zinc absorption. Symptomatology responds dramatically to the administration of increased amounts of zinc given by mouth.

10.6.2 Transcobalamin 2 deficiency. Autosomal recessive defects in the vitamin B_{12} transport protein, transcobalamin 2, have been described. These defects impair the normally rapid cell proliferation required for haematopoiesis, lymphocyte proliferation and gastrointestinal tract epithelial cell regeneration. Affected infants present with diarrhoea, failure to thrive, megaloblastic anaemia, defective granulocyte function and immunodeficiency involving primarily B lymphocyte function. Administration of vitamin B_{12} in pharmacological doses rapidly reverses the signs and symptoms. Folinic acid may also be required.

10.6.3 Type I hereditary orotic aciduria. An autosomal recessive disease that presents with retarded growth, recurrent diarrhoea, megaloblastic anaemia, increased numbers of infections (including fatal meningitis and varicella), and lymphopenia with decreased numbers of T lymphocytes and impaired cell-mediated immunity.

10.6.4 Intractable diarrhoea, abnormal facies, trichorrhexis and immunodeficiency. Several patients have been reported with prenatal growth retardation, facial dysmorphism with hypertelorism, woolly, friable hair (trichorrhexis) and severe secretory diarrhoea. While serum immunoglobulin was normal, antibody responses were defective. *In vitro* lymphocyte responses to mitogens were likewise normal, but skin tests for delayed hypersensitivity (DTH) were diminished. Stankler Syndrome may be similar.

10.6.5 Methylmalonic acidaemia. Methylmalonic acidaemia is similar to Transcobalamin II deficiency; it represents a series of several distinct enzymatic defects that affect cobalamin (B_{12}) metabolism and result in the accumulation of excess levels of methylmalonic acid, which inhibits bone marrow stem cell growth. Pancytopenia is common; B-cell numbers and serum IgG may be reduced. There may be no response to vitamin B_{12}. Folic acid treatment may reverse the problem.

10.6.6 Biotin dependent carboxylase deficiency. Infants affected with this autosomal recessive condition present with convulsions, ataxia, alopecia, *Candida* dermatitis, keratoconjunctivitis and increased urinary excretion of beta-hydroxyproprionic acid. Isolated IgA deficiency and reduced numbers of peripheral T and/or B lymphocytes have been reported. Biotin administration results in biochemical and clinical improvement.

10.6.7 Mannosidosis. This lysosomal storage disease resembles Hurler syndrome with abnormal facies, dysostosis, hepatosplenomegaly and recurrent infections. The accumulation of the mannose-rich lysosomes may interfere with both neutrophil and lymphocyte function.

10.6.8 Glycogen storage disease, Type 1b. Patients with this variant of glycogen storage disease may have neutropenia and neutrophil dysfunction, presumably due to defective glucose metabolism. They have recurrent infections.

10.7 Hypercatabolism or loss of immunoglobulin

Many diseases are associated with hypercatabolism of Ig. These can be distinguished from failure of Ig production by metabolic studies. The following are some of the conditions in which hypercatabolism of Ig may lead to immunodeficiency.

10.7.1 Familial hypercatabolism. A kindred has been described with recurrent infections, bone abnormalities, abnormal glucose metabolism and diminished levels of serum albumin and immunoglobulin which could not be explained by increased gastrointestinal or urinary losses.

10.7.2 Intestinal lymphangiectasis. Losses of lymphocytes and immunoglobulins into the gut can result in significant lymphopenia, diminished cell-mediated immunity and decreased serum Ig levels.

10.8 Other

10.8.1 Hyper–IgE syndrome (Job syndrome). Recurrent (usually staphylococcal) abscesses that are often 'cold', lung abscesses which result in pneumatocoeles, skeletal anomalies, coarse facies, eosinophilia and very high serum levels of IgE are characteristic of the Job or hyper-IgE syndrome. The B lymphocytes from these patients spontaneously produce large amounts of IgE *in vitro*. Several kindreds involving both males and females, and affected mothers or fathers with affected children, have been reported, suggesting that in some instances the disease is autosomal dominant. Sporadic cases also occur. The immunological defect is not yet fully understood.

10.8.2 Chronic muco-cutaneous candidiasis. These patients have severe persistent candidal infections of skin and mucosa. They have markedly impaired cell mediated immunity to *Candida* antigens. The relationship, if any, between the specifically diminished T-lymphocyte responses and infection is not understood. A mannose deficiency has been noted in the monocytes of some patients. This condition is frequently associated with familial polyendocrinopathy. Severe secondary bacterial infections may supervene.

10.8.3 Hereditary or congenital hyposplenia or asplenia. People with hyposplenia or asplenia (whether post-traumatic, surgical, congenital or hereditary) are at increased risk for sepsis. Infants with congenital or hereditary asplenia are particularly prone to such infections. The responsible organisms are usually pyogenic, pneumococci being the most common. Increased susceptibility to intracellular parasites (for example, malaria) and some viral agents has also been reported.

10.8.4 Ivemark syndrome. This syndrome probably represents disturbed laterality including partial *situs inversus* during very early embryogenesis resulting in major defects in organ formation. In addition to cardiac defects, asplenia is found. The problems of infection are as described in Section 10.8.3.

10.8.5 Familial intestinal polyatresia. Multiple areas of atresia throughout the gastrointestinal tract characterize this AR syndrome. Several patients have had an associated severe combined immunodeficiency, all with markedly decreased immunoglobulin and variably decreased T-cell number and function, and reduced immunoglobulins. Patients with familial intestinal polyatresia require surgery; as these patients may have an underlying SCID, all blood given must be irradiated to prevent engraftment.

10.8.6 Immunodeficiency with thymoma. About 10% of patients with thymoma have an immunodeficiency, an association described in 1954. The disorder is seen most commonly in older (> 40 years) adults and is generally identified by the radiological finding of the thymoma and on subsequent study by hypogammaglobulinaemia. The immunodeficiency often initially appears similar to common variable immunodeficiency (Section 9.3.7). The immunodeficiency may precede the thymoma. The types of infections (candidiasis, herpes), the frequent occurrence of autoimmune disease (myasthenia gravis, pernicious anaemia, diabetes, thrombocytopenia) and studies of cellular responses indicate that T cells as well as B cells are affected; the condition thus fits the criteria for a combined immunodeficiency (see Section 9.2). The occurrence is sporadic and affects both sexes. No familial cases

have been reported. Immunoglobulin administration decreases the number of infections. Surgical removal of the thymoma may meliorate some symptoms, but does not cure the immunodeficiency. If the thymoma is malignant, death usually follows within a few years of diagnosis.

11 COMPLEMENT DEFICIENCY

11.1 Complement system

The classic complement system consists of nine numbered components (Cl to C9) and five regulatory proteins (Cl inhibitor, C4 binding protein, properdin, Factors H and I). The first component (Cl) comprises three subcomponents, Clq, Clr, and Cls. It is the molecular interaction between Clq and aggregated IgG or IgM (as in an antigen–antibody complex) that initiates activation of the classical complement sequence. The fixation of Clq activates Clr and Cls. Cls cleaves C4 and C2, whose active fragments C4b and C2a form the classical pathway C3 convertase.

An alternative pathway to C3 activation consists of C3b, Factor B and Factor D. Factor B binds to a cleavage fragment of C3, C3b, to form C3bB. Factor D cleaves the bound Factor B to form the alternative pathway C3 convertase (C3bBb). It activates C3 in a fashion similar to the C3 convertase of the classical pathway, C4b2a. Properdin appears to stabilize this alternative pathway C3 convertase.

A large number of biological activities important in the inflammatory response and in host resistance to infection take place at various steps in complement activation. The lytic property for bacterial or animal cells, however, requires the activation of C5 to C9 by the classical or alternative pathway. The enhancement of phagocytosis by complement is probably of great biological significance and requires the deposition of C3b or iC3b on the particle to be ingested. Certain viruses are neutralized after interaction with antibody and only the first two complement components (Cl and C4); other viruses require C2 and C3 in addition. Immune adherence, a property whereby antigen–antibody complexes adhere to complement receptor 1 (CR1), occurs with complement activation through the C4 and C3 steps. The ligands for CRl are C4b and C3b. Histamine release from mast cells, smooth muscle contraction and increased vascular permeability caused by anaphylatoxin activity are properties of each of the two small fragments (C3a and C5a), which are released when C3 or C5 are cleaved by their respective convertases. These fragments are also chemotactic for polymorphonuclear leucocytes, particularly C5a, which also causes exocytosis of neutrophils. The classic complement pathway appears to be important in the dissolution of immune complexes.

Complement enhances B-cell activation at two distinct stages of development within the lymphoid compartment: (a) formation of the PALS (periarteriolar lymphoid sheath)-associated foci; and (b) survival within the germinal centre (GC). The former involves reduction in the threshold of B-cell signalling via the CD21/Tapa-1 complex; the latter involves increased adhesion between the B cells and the follicular dendritic cells, mediated by CD21 and C3d-antigen in germinal centre interactions.

11.2 Genetic defects in human complement

Genetic defects have been described for almost all the complement components in humans, including Clq, Clr (and Cls), C4, C2, C3, C5, C6, C7, C8 and C9 deficiency (Table 6). In all these instances defects are transmitted as phenotypically autosomal-recessive traits, and the heterozygotes can usually be detected because their sera contain approximately half the normal level of the deficient component as determined by functional and/or immunochemical tests. Non-functional variants of Clq have been described. C8 deficiency is unusual in that the β chain is not covalently associated with the α and γ chains. Thus, affected Caucasian C8 deficients lack the β chain and Black C8 deficients lack the α, γ chains. Both forms have nonfunctional, incomplete C8 molecules in their serum. C9 deficiency has a very high incidence in the Japanese. Genetic deficiencies in the alternative pathway are very rare. Deficiency of properdin is X-linked. The mode of inheritance of Factor D deficiency is not entirely clear.

Genetic defects have also been recognized for three inhibitors of the complement system: Cl inhibitor, Factor I and Factor H. Deficiency of the Cl inhibitor is inherited as an autosomal dominant. This deficiency is associated with hereditary angioedema (HAE), or Quincke's disease. In l5% of affected kindred the sera contain normal or elevated amounts of an immunologically cross-reacting (CRM+), nonfunctional protein due to missense point mutations in the C1 inhibitor gene in the exon encoding the active site. In the majority of affected kindred the defects are due to nonsense mutations or unequal crossovers in the Alu sequences of introns 4, 5, 6, 7 and 8.

The genes for factor B, C2 and C4 are located on the short arm of chromosome 6 between HLA-D and HLA-B. The C4 gene is duplicated and the two genes are designated *C4A* and *C4B*; C4A molecules usually bear the Rodgers blood group substance and C4B the Chido blood group substance. Complete C4 deficiency is very rare and occurs only when all four alleles (the two alleles of C4A and the two of C4B) are not expressed. In one case, this was due to isodisomy of a paternal chromosome 6p that was deficient in *C4A* and *C4B* (C4AQO and C4BQO). Thirty-five per cent of individuals in all racial groups lack one to three C4 alleles. Those with C4AQO have a high incidence of SLE and juvenile rheumatoid arthritis. The genes for factor B and C2 are so tightly linked that no crossover has yet been observed between them, but unequal crossover in the MHC may result in the expression of three C4 A alleles and one C4B allele, or vice versa.

Genetic polymorphisms are known for C4A, C4B, C2, Factor B, C3, C6, C8α, and C8β. Polymorphic variants of C5, C7, Factor D, Factor H, Factor I and C1 inhibitor are rare.

All patients with complement deficiency are more or less unduly susceptible to infection and to development of immune complex disease. For example, patients with Cl inhibitor deficiency (HAE) have prominent angioedema but are also prone to develop immune complex disease.

Impeded androgens have proved extremely effective in the treatment of hereditary angioedema. Purified Cl inhibitor preparations are available for intravenous administration and should be used in the treatment of acute attacks of angioedema. There is no satisfactory replacement therapy for the other complement deficiencies, largely because the catabolic rate of these proteins is very high. Sometimes patients with late component deficiencies require antimicrobial prophylaxis or immunizations because of recurrent neisserial infections.

12 DEFECTS OF PHAGOCYTE NUMBER AND FUNCTION

Apart from congenital neutropenia, which has several causes, there may be inherited defects of phagocyte function, affecting polymorphonuclear and/or mononuclear phagocytes. Neutrophil function depends on movement in response to chemotactic stimuli,

adherence, endocytosis, and killing or destruction of the ingested particles. Mobility depends on the integrity of the cytoskeleton and the contractile system; directional mobility is receptor mediated. Endocytosis depends on the expression of certain membrane receptors, for example, for IgG, C3b and iC3b, and on the fluidity of the membrane. Congenital defects of phagocyte number and/or function and their associated features are listed in Table 5.

Assays for chemotaxis can be performed by the use of Boyden chambers or migration under agarose; defects of contractibility, however, can only be assessed with Boyden chambers. The measurement of nitroblue tetrazolium (NBT) dye reduction by actively phagocytosing leucocytes has been accepted as a standard measure for the adequacy of superoxide production. Better suited for detection of partial defects are more sensitive assays including chemiluminescence and the direct measurement of superoxide. Assays for bacterial killing are demanding and yield highly variable results depending on the bacterial species used in the assay.

12.1 Congenital neutropenias

12.1.1 Severe congenital neutropenia (SCN, Kostmann syndrome). SCN is characterized by profound neutropenia ($< 200/\mu l$) and a maturation arrest of myeloid progenitor cells at the promyelocyte–myelocyte stage. Pharmacological doses of G-CSF (ranging from 0.8 to 70 μg/kg/day) increase the neutrophil count in the majority of patients and decrease infections (mainly otitis, stomatitis and pneumonia) significantly.

While most SCN patients have normal G-CSF receptors, a subgroup presents with nonsense G-CSF receptor mutations truncating the C-terminal cytoplasmic region crucial for maturation signalling. Patients in this subgroup develop myelodysplastic syndrome and acute myeloid leukaemia. They should not be treated by G-CSF, but probably by bone marrow transplantation if a donor is available.

12.1.2 Cyclic neutropenia. Cyclic neutropenia is characterized by 21-day oscillations of blood counts with neutrophil levels fluctuating between the lower limit of normal and zero. During the periods of severe neutropenia patients are prone to severe infections, but otherwise are relatively well. G-CSF in doses of 1–5 μg/kg/day raises the oscillating neutrophil counts and shortens the periodicity of the cycles which persist. None of the patients developed MDS/AML.

12.2 Defects of motility

12.2.1 Leukocyte adhesion defects (LAD). A large number of cases has been described with a defect in the iC3b receptor of phagocytes (CD11b), the C3 dg receptor of phagocytes called pl50,95 (CD11c) and the LFA-l (CD11a) adhesion molecule of T lymphocytes, NK cells and phagocytes. This deficiency results from abnormal biosynthesis of a 95-kDa β chain (CDl8), which is common to the iC3b receptor, pl50/95 and LFA-l; the gene encoding the beta chain maps to chromosome 21. This defect has been called leucocyte adhesion defect type 1 (LAD1). It is inherited as an autosomal recessive disorder. The phenotypic expression of the leucocyte adhesion defect is variable. In the severe phenotype <l% of normal adhesion molecules are expressed whereas in the moderate phenotype up to l0% of these molecules are expressed. Patients have defects in mobility, adherence and endocytosis. They usually present with infections of skin, periodontitis and intestinal or perianal fistulas. In the severe phenotype omphalitis, delayed umbilical cord separation, septicaemia and massive leucocytosis (up to 100 000 mm^3) are typical.

A second type of leucocyte adhesion deficiency (LAD2) has been described in unrelated Palestinian children. These infants are unable to synthesize fucose from GDP mannose so that they cannot form the Sialyl-Lewis x ligand for the selectin molecules. The phenotype in this form of LAD is similar to the common form of LAD except that short stature, mental retardation as well as Bombay (hh) blood group has been noted in the former. It is inherited as an autosomal recessive. The enzyme defect has not been precisely defined and its chromosomal location is not yet known.

12.2.2 Specific granule deficiency. Neutrophils have two types of granules which contain a variety of enzymes. In a few patients described to have abnormal neutrophil structure (bilobed nuclei), specific (secondary) granules (which normally contain lactoferrin and receptors for chemotactic factors as well as for iC3b and C3 dg) are incompletely formed. Defective chemotaxis has been described. Clinically there are increased numbers of skin infections and progressive pulmonary disease. The precise nature of the defect is unknown.

12.2.3 Shwachman syndrome. Hereditary pancreatic insufficiency associated with neutropenia, defective neutrophil mobility and chemotaxis, thrombocytopenia and anaemia and often metaphyseal chondrodysplasia with short stature are the principal features of this syndrome. Affected infants have recurrent pyogenic sinopulmonary and skin infections and may have hypogammaglobulinaemia. It is inherited as an AR.

12.2.4 Other. Phagocyte function may also be defective in a number of generalized hereditary diseases, such as glycogen storage disease type Ib. The phagocytic dysfunction does not constitute a characteristic or diagnostic feature of these diseases. Certain immunodeficiency syndromes may be associated with a constant primary (e.g. the Wiskott–Aldrich syndrome) or a fluctuating secondary chemotactic defect.

12.2.5 Treatment. Infections in phagocyte deficiencies should be treated with appropriate antibiotics, surgery and, in the case of septicaemia, neutrophil transfusion. In the case of defects of intracellular killing, lipophilic antibiotics able to penetrate phagocytes are preferable. Long-term infection prophylaxis by sulphamethoxazole-trimethoprim (antibacterial) and itraconazole (antiaspergillus) is valuable, especially for CGD. If neutrophils are transfused, cells should be mobilized in the donors by G-CSF, and should be protected in the recipient from apoptosis by G-CSF as well. Patients with congenital neutropenias (e.g. SCN, cyclic neutropenia) and the neutropenia associated with the hyper-IgM syndrome profit from G-CSF treatment. G-CSF must not be given to a subgroup of SCN having G-CSF receptor mutations predisposing to MDS/AML.

Some CGD patients (e.g. with mutations still allowing residual O_2- production) may profit from interferon γ treatment. Bone marrow transplantation has been successful in patients with SCN, LAD, CHS and CGD.

12.3 Defects of microbial killing

12.3.1 Chronic granulomatous disease (CGD). Defects in intracellular killing of ingested microorganisms usually result from failure of production of superoxide anion, singlet oxygen, and hydrogen peroxide. This failure results in chronic granulomatous disease (CGD). The organisms cultured from lesions of patients with CGD are generally catalase-producing and include Staphylococci, *Escherichia coli*, *Serratia marcescens*, Nocardia, fungi, such as *Aspergillus*, and other organisms with formation of

chronic infected granulomas, especially of lymph nodes, liver, lung and gastrointestinal tract. The reaction $NADPH + 2O_2 \rightarrow NADP + 2O_2- + H^+$ requires NADPH oxidase, a multicomponent enzyme localized in the plasma membrane of phagocytic leucocytes. The enzymatic unit of NADPH oxidase consists of a flavoheme protein called cytochrome b558, a heterodimer composed of a 91-kDa chain (gp91phox) and a 22-kDa chain (p22phox). When phagocytes are activated, a number of cytosolic oxidase components translocate to the membrane and induce enzymatic activity by a conformational change in the flavocytochrome. X-linked CGD results from a defect in gp91phox. In some cases, CGD is associated with a defined deletion in the short arm of the X chromosome at Xp2l. In some cases of autosomal recessive CGD, p22phox, whose gene is encoded on chromosome 16, is defective; in other cases one of two cytosolic components of NADPH oxidase, p67phox or p47phox, is defective.

12.3.2 Neutrophil G6-PD deficiency. Glucose-6-phosphate dehydrogenase (G6-PD) is a necessary component of the hexose monophosphate shunt. The G6-PD gene, located at Xq28, is prone to frequent mutations; over 200 variants have been recorded. In neutrophil G6-PD deficiency the variant leads to a severely defective enzyme and, because of its function in NADPH generation, results in reduced intracellular H_2O_2 production on leucocyte activation. As in CGD, there is failure in the killing of catalase-positive intracellular organisms. The clinical presentation is the same as in CGD except that it occurs at a later age. As NBT cannot be reduced, the NBT test can be used for ascertainment. Reduced G6-PD in red blood cells causes concomitant chronic haemolytic anaemia.

12.3.3 Myeloperoxidase deficiency. Myeloperoxidase is one of the more abundant enzymes in polymorphonuclear leucocytes. The gene is located at 17q22–23. Deficiency is not uncommon (1:2000–4000 in the USA) and is usually clinically silent. Granulocytes lacking the enzyme fail to kill *Candida*; some affected people (presumably having a more defective mutation and often in association with other diseases) have suffered from severe, recurrent candidal infections.

12.3.4 Leucocyte mycobactericidal defects (LMD). A number of patients have recently been described who developed disseminated disease due to mycobacterial species that are not ordinarily pathogenic in humans as well as systemic disease from Bacille Calmette-Guárin vaccination. The only other associated infections in these patients are due to *Salmonella* species, which are encountered in about 50% of patients with LMD.

The most severe phenotype of LMD is observed in patients with complete deficiency of the interferon-γ R1; these patients are incapable of forming granulomas in response to mycobacterial infections. Missense mutations in this receptor result in a less severe phenotype. A comparable severe phenotype was discovered in a child with complete deficiency of interferon-γ R2. Interleukin-12 is a heterodimer of p35 and p40 subunits. A mutation in the p490 subunit of IL-12 has been discovered and results in a mild phenotype of LMD, i.e. granulomas are formed in the presence of mycobacterial infection and the patients respond to interferon-γ therapy. The inheritance of this defect in the one kindred well studied is not clear. Several patients with a defect in the β1 chain of the IL-12 receptor have been described to have a mild phenotype.

[a]These determinations require special facilities and can be arranged by writing to Dr H. D. Ochs, Department of Paediatrics RD-2O, University of Washington, Seattle, WA 98195, USA.

[b]Obtainable from the Institute Pasteur-Merieux, Lyon, France.

[c]Antigen and assay obtainable from Dr M. Eibl, Institute of Immunology, Borschkegasse 8a, 1090 Vienna, Austria.

[d]Obtainable from Merck, Westpoint, PA 19486, USA.

[e]Obtainable from Hollister-Stier Labs, Box 3145, Terminal Annex, Spokane, WA, USA.

[f]Undiluted glycerin-free Dermatophytin (Trichophyton), Hollister-Stier Labs, Box 3145, Terminal Annex, Spokane, WA, USA.

[g]Mumps skin test, Eli Lilly & Co, Indianapolis, IN 46206, USA.

[h]Paediatric diphtheria and tetanus toxoid, Wyeth Laboratories, PO Box 8299, Philadelphia, PA 19101, USA.